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# Isolation and Identification of Pathogenic Bacteria from Fresh Fruits and Vegetable Sold in Market of Janakpurdhm

Nagendra Prasad Yadav<sup>1\*</sup> | Rakesh Kumar Yadav<sup>2</sup> | Suveksha Sah<sup>3</sup> | Harinarayan Purbe<sup>4</sup>

1Department of Microbiology, Janaki Medical College, Janakpur, Tribhuvan University, Nepal

2Department of Pharmacology, Pakalihawa Campus, IAAS, Tribhuvan University, Nepal

3Department of Microbiology, Model Multiple College, Tribhuvan University, Janakpur, Nepal

4Department of Emergency Medicine, Janaki Medical College, Tribhuvan University, Janakpur, Nepal

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\*Correspondence:

[nagendrayadav2073@gmail.com](mailto:nagendrayadav2073@gmail.com)(NPY)

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## Abstract:

**Introduction:** Vegetable and fruits are highly nutritious palatable food. They are enriched sources of vitamins mineral and fibres. They are the essential part of our diet and used all over the world daily. They are perishable food. The microorganisms get easy access to enter inside it. They get contaminated with different types of bacteria, during cultivation, harvesting, processing, transportation, storage and consumption. Eating of bacterial contaminated fruits and vegetable can lead to severe problem to the community people. Bacterial contamination can cause degradation, loss of quality and potential health risk to the consumer.

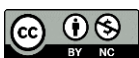
**Materials and Methods:** A total of 70 fresh fruits and vegetable were collected from different retailer of Janakpur metropolitan city, aseptically. Bacterial count and their identification were done in the microbiology lab in Model Multiple College Janakpur using different media and biochemical reagents.

**Results:** Among them *Pseudomonas species*, *Escherichia coli*, *Lactobacillus species*, *Staphylococcus species* and *salmonella species* were the dominant bacteria. All of the fruits and vegetable were found heavily contaminated with bacteria. Bacterial count in different sample were found as in Guava count was  $3.1 \times 10^4$  CFU/ml, Apple was  $4.3 \times 10^4$  CFU/ml, Tomato  $6.2 \times 10^4$  CFU/ml, Cucumber  $5.0 \times 10^4$  CFU/ml, carrot was  $1.3 \times 10^4$  CFU/ml, Amara was  $8.5 \times 10^4$  CFU/ml and Orange was  $1.2 \times 10^4$  CFU/ml.

**Conclusions:** The fruits and vegetable available in Janakpur market is highly contaminated and has a great risk for consumer health.

## Keywords

Biochemical, Contamination, Culture media, Disease



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## INTRODUCTION

Food contamination is a critical issue in this year. In recent years, outbreaks of human infections associated with the consumption of contaminated food have increased, despite their nutritional and health benefits [1,2]. Food contamination refers to the presence of harmful bacteria or substances in food that can cause illness or spoilage. There are different types of food contaminations, biological contamination, chemical contamination, physical contamination. Biological contamination is the presence of harmful microorganisms such as bacteria, viruses, parasites and fungi in food. The common bacterial contaminants are *Escherichia coli*, *Salmonella*, *Pseudomonas*, *Staphylococcus*, *Campylobacter*, *Listeria* etc [3-5]. Contamination in a food can occur at any stage of the production processing, from farm to the table. Microbial contamination of fruits and vegetables is a complex process influenced by various factors, water content, pH level, storage condition, physical damaging etc [6-8]. These all factors make the fruits and vegetable very prone towards the bacterial growth. Microbial contamination of fruits and vegetable is a common problem that occurs when microorganisms such as bacteria, yeast, and molds grow on or within [7,9]. Microbial contamination in fruits and vegetable not only cause loss of quality, it can cause spoilage of food [10,11]. Consumption of such spoiled fruits and vegetable is the source of food borne diseases and intoxications. The major food spoilage microorganisms are bacteria, yeast and molds. Bacteria are the most common contaminants of food [12-14].

Natural characteristics of fruits and vegetable favour the microbial growth. Microbial growth in food can cause alteration in the physical and chemical properties of food [15,16]. Microbial contamination is commonly exposed to fruits and vegetables by contact with dirt, dust, water and by handling during harvesting or post harvesting processing [17-19]. Therefore, a wide range of microorganisms are present including plant pathogen and human pathogen. Due to lack of surveillance and inadequate screening of these raw fruits and vegetables, most of the outbreaks have become undetected and there is very little information available in the literature. In another study, it was found that bacterial contamination is the major cause of food spoilage in Nepal. A major contributor factor of contamination is the use of untreated water and manure as fertilization in the production of fruits and vegetables [19,20]. In developing countries like Nepal, both poverty and poor sanitation are commonly correlated with food contamination. The ingestion of such types of contaminated fruits and vegetable is related to many outbreaks of human gastroenteritis.

## MATERIALS AND METHODS

### **Study design and setting:**

This was a cross-sectional type study conducted in Janakpur Nepal, between February 2024 to the April 2024. Samples were collected from major market areas such as Sita Chowk and Janaki Chowk in Janakpur Metropolitan city.

### **Sample collection:**

Seventy samples of fresh fruits and vegetables were aseptically collected in a sterile polythene bag and transported to the laboratory in insulated ice boxes to prevent contamination.

### **Sample preparation:**

Within an hour of collection, the samples were taken to the laboratory and rinsed for each with sterile distilled water, then diluted 10-fold serially. After washing the 10ml washed aqueous suspension of each sample was mixed with 90ml Nutrient broth and incubated for 24 hours at 35 degrees centigrade. This overnight culture in Nutrient broth was subcultured into different selective media for the isolation and identification by streak plate method.

**Bacterial enumeration:** The spread plate method was used on Plate Count Agar to determine the number of colony forming units (CFUs). Isolation and identification of bacteria: One loopful culture from Nutrient broth were streaked on the surface of different selective media using MacConkey agar (for *E. coli*), Thiosulfate Citrate Bile sucrose agar (for *Vibrio*), Xylose Lysine Deoxycholate agar (for *Salmonella*), Manitol Salt agar (for *Staphylococcus*), Cetrimide agar (for *Pseudomonas*). Plates were incubated at 35 degrees centigrade for 24 to 48 hours.

### **Data management and Statistical Analysis:**

Data were analysed to determine bacterial prevalence and CFU counts. Descriptive statistics were applied.

### **Ethical Considerations:**

This study was conducted following ethical guidelines, ensuring no harm to individuals or the environment. No institutional review board approval was necessary as it involved non-human subjects.

## **RESULTS:**

In this study, all samples of fruits and vegetables were found contaminated. Table 1 represents the total bacterial count in fruits and vegetables sampled. The microbial burdens of different samples were *E. Coli*, > 1100 CFU/100ml. Bacterial count was found as in Guava count was  $3.1 \times 10^4$  CFU/ml, Apple was  $4.3 \times 10^4$  CFU/ml, Tomato  $6.2 \times 10^4$  CFU/ml, Cucumber  $5.0 \times 10^4$  CFU/ml, Carrot was  $1.3 \times 10^4$  CFU/ml, Amara was  $8.5 \times 10^4$  CFU/ml and Orange was  $1.2 \times 10^4$  CFU/ml.

Table 2 represents the species of bacteria isolated from the fruits and vegetables. Six species of bacteria were

**Table 1 | Fruits and vegetables with their total bacterial count**

Fruits/ Vegetable	Total sample tested	Total bacterial count
Guava	15	3.1 x10 <sup>4</sup> CFU/ml
Apple	10	4.3x10 <sup>4</sup> CFU/ml
Tomato	15	6.2x10 <sup>4</sup> CFU/ml
Cucumber	10	5.0x10 <sup>4</sup> CFU/ml
Amara	10	8.5x10 <sup>4</sup> CFU/ml
Orange	10	1.2x10 <sup>4</sup> CFU/ml

**Table 2 | Bacterial Isolates from different fruits and vegetables**

S.N.	Biochemical test and result of Gram-negative bacteria				
	Bacteria	Indole test	MR test	VP test	Citrate test
01	<i>E. coli</i>	Positive	positive	Negative	Negative
02	<i>Vibrio</i>	positive	negative	Negative	Positive
03	<i>Salmonella</i>	negative	positive	Positive	Negative
04	<i>Pseudomonas</i>	Negative	negative	Negative	Positive
S.N.	Biochemical test for Gram positive bacteria				
	Bacteria	Catalase test	Coagulase test		
05	<i>Lactobacillus</i>	Positive	Negative		
06	<i>Staphylococcus</i>	Positive	Positive		

isolated from each of the sample and they were identified on the basis of the cultural, morphological and biochemical characteristics. Both Gram positive and Gram-negative bacteria were found which was further separated and identified by using following biochemical test.

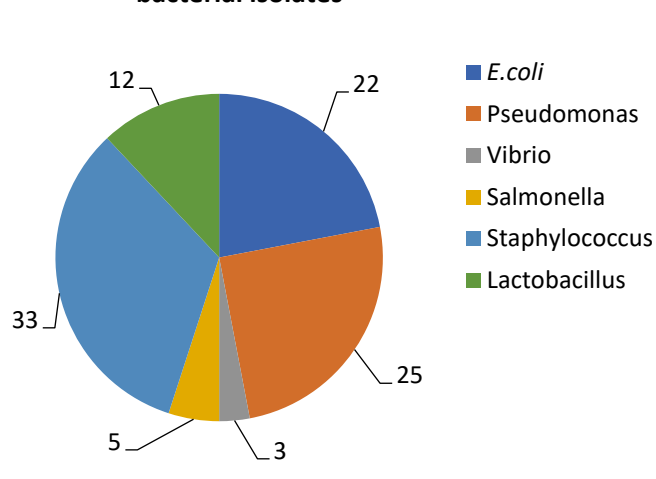
**Figure 2 | Bacteria percentage in the bacterial isolates**

Figure 1 represents bacteria percentage in the bacterial isolates. Opaque colonies from tomato juice agar (TJA) plate were *Lactobacillus* and golden yellow colonies from manitol salt agar (MSA) plate with catalase and coagulase positive were *Staphylococcus*. Among the bacterial isolates, *Pseudomonas* species (25%), *E. Coli* (22%), *Vibrio* species (03%), *Salmonella* species (05%), *Staphylococcus aureus* (33%) and *Lactobacillus* species (12%) were found.

## DISCUSSIONS

The widespread, bacterial contamination found in fresh fruits and vegetable pose a serious public health risk. The presence of *Staphylococcus*, *E. Coli*, and *Pseudomonas* are linked to food borne illness- highlights the potential for community outbreak of disease like gastroenteritis. Contamination likely occurs at various points from farm to market, compounded by the use of untreated water and poor sanitation in some areas. Consistent monitoring and public awareness are necessary to reduce contamination risk. All the samples were found contaminated with bacteria and major contaminants were *Staphylococcus*, *Escherichia coli* and *Pseudomonas*. These bacteria bear public health concern and common pathogen in community of poor personnel hygiene condition [14]. This is the serious health concern for the people of Janakpur. Fruits and vegetable acquired bacteria from the different source of processing and storage. From cultivation in farm, harvesting, post harvesting, transportation and distribution fruits and vegetable become contaminated with pathogenic bacteria [16,17]. The presence of pathogenic bacteria in such common food is one of the most important methods of disease transmission. Most of the diarrhoea and dysentery detected in our community is food born and mainly due to ingestion of such type of contaminated fruits and vegetable. Necessary steps should be taken as early as possible to avoid contamination in such food [11]. This study reflects the poor hygienic condition of fruits and vegetable sold in Janakpur market. Health and food security are the

matters of government responsibility [20]. It is also the responsibility of the government to ensure the right to healthy food, health and social security provided by the constitution of Nepal.

#### ADDITIONAL INFORMATION AND DECLARATIONS

**Acknowledgment:** The authors are grateful for the cooperation of the fruit and vegetable vendors, whose help for providing the sample was invaluable in this research.

**Competing Interests:** The authors declare no conflicting interests to disclose.

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**Author Contributions:** Concept and design: NPY and SS, Statistical analysis: NPY, Writing of the manuscript: NPY,

**CONCLUSIONS** The finding indicates high levels of bacterial contamination in fresh product in Janakpur, understanding the need for improved hygiene practices. Regular testing, market monitoring, and training for vendors on safe handling practices are recommended to protect consumer health.

RKS & SS. Data collection: NPY and SS, Revision and editing: NPY, RKY, SS and HP. All authors have contributed equally for the concept and design, statistical analysis, writing of the manuscript, data collection, revision and editing. All authors have read and agreed with the contents of the final manuscript towards publication.

**Data Availability:** Data will be available upon request to corresponding authors after valid reason.

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