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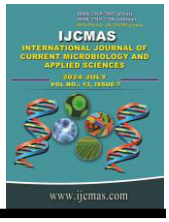

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Isolation of Bacterial Diversity from the Surface of Fresh Fruit Samples Available in Local Market of Ayodhya City, India

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A B S T R A C T

Fruits are food we eat every day. It is usually listed as an entire fruit, juice, beverage, or still drink, etc., on diet charts worldwide. Fruit is a great source of key nutrients such as vitamins, minerals, and antioxidants that help prevent diabetes, cancer, and heart disease. The demand for food that is microbiologically safe has been highlighted in recent years due to rising consumer awareness. In this research primary goal was to isolation of microorganisms from fresh fruit samples and to specify enumeration of bacteria in fresh fruit available in Ayodhya market, Uttar Pradesh, India.

Introduction

Fruit and other fresh goods are staples of the human diet and are consumed in great amounts in the majority of civilizations. Fresh fruits are vital for human nutrition and there is strong evidence that eating them has positive effects on one's health and nutrition (Shakir *et al.,* 2009). Fruits that have been improperly washed might harbour germs that can cause contamination and sometimes contaminated water is sprinkled on the fruits which contains coliform bacteria. Because they are easily found and linked to faecal contamination, indicator bacteria like faecal coliforms and water bodies' microbiological health is often assessed using *Escherichia coli*, or *E. coli*. It is interesting that the presence of indicator bacteria in water implies that faecal contamination has occurred, even if these bacteria may not always indicate the presence of dangerous microorganisms, such as viruses and bacteria that can cause illnesses including cholera, typhoid fever, and gastroenteritis (Kiran *et al.,* 2023). Furthermore, the use of unsanitary water preservation methods without refrigeration, unsanitary environments frequently containing swarming fruit and house flies, and airborne dust can all serve as sources of infection. Fruits are frequently subjected to microbial contamination through handling during harvest or during post-harvest processing, as well as through contact with dirt, dust, and water. The use of manure and untreated waste water as fertilizers for fruit cultivation is a significant cause of pollution.

Fruit, which includes seeds and is often juicy, sweet, and edible raw, is a structural component of plants. Examples of fruits with seeds include oranges, bananas, mangoes, apples, and grapes. They are seed-containing carpels or the right ovaries (MC Gee *et al.*, 2004). Comparable to fruits, they are composed primarily of water (85% on average), some fat, different phytochemical substances,

and carbohydrates (cellulose and starch) in trace amounts (Ihekoronye and Ngodsy *et al.,* 1985). The majority of fruits are used for dessert, and they may also be processed to create liquid products like wines, fruit juices, and jams, jellies, and other concoctions. Fruit products are sold in tetra-packet packaging, in cans, and in bottles.

Oranges are high in potassium, folic acid, and vitamin C, which are all good sources of phytochemicals and bioavailable antioxidants (Franke *et al.,* 2004). They also considerably improve blood lipid profiles in those with hypercholesterolemia (Kurowska *et al.,* 2000). Numerous additional plant chemicals and antioxidants found in oranges have the potential to lower inflammation and fight illness. Oranges are a great way to get vitamin C, an antioxidant, and they may also help prevent the creation of free radicals, which are linked to cancer. Keeping the permitted salt intake constant is crucial for reducing blood pressure. But increasing one's consumption of potassium could be just as crucial for lowering one's chance of developing high blood pressure. Because it may be facilitate the blood vessels' opening and relaxing.

At least two servings of fruit should be consumed daily by adults, according to USDA guidelines (United States Department of Agriculture, 2005). Numerous research investigations have documented the positive impact that fresh fruits have on human health. For example, consuming more fruits lowered the risk of cancer by 19% (Van't Veer *et al.,* 2000).

Intense efforts are being made to control the issues and enhance food safety due to an increase in food-borne illnesses linked to fresh fruits globally (Denis *et al.,* 2016). A multitude of scientifically researched and implemented solutions are available for managing harmful microorganisms in fresh fruits. According to Park *et al.,* (2001), a variety of chemical agents, including hydrogen peroxide and chlorine, have been used as sanitizers to clean the surfaces of fresh produce.

All around the world, eating fresh fruit is an essential part of a healthy diet. Food habits grew more varied and patterns of food intake shifted from food for hunger to food for health and well-being as the nation got more urbanized throughout time. Households in both rural and urban areas have clearly changed in their patterns of consumption. Another important element influencing the shift in purchasing habits is the growing urbanization of the population. Food habits have drastically changed as a result of globalization, according to a recent analysis by NCAER (2014). India's consumption over the years. With an annual production of over 94 metric million tons, India ranks second in the world for fruit production (Gouri- Sundaram *et al.,* 2006). Owing to its diverse climate, India is among the select few nations that cultivates nearly exclusively tropical and exotic fruits. As a result, foods like fruit salads, sprouted seeds, and minimally processed fruits are becoming more and more popular. A healthy low-fat diet rich in fruits led to a substantial 40% decrease in cardiac issues and 45% reduction in mortality after one year when compared to a typical low-fat diet, according to Singh *et al.,* (1992) analysis of the Indian Experiment of Infarct Survival (IEIS) data. The aforementioned study is only one of several that highlight how important fruit is to a healthy diet. Numerous comprehensive reports on the frequency of foodborne outbreaks worldwide have been published. According to a CDC study, throughout the ten-year period from 1973 to 1988, the number of breakages related to fresh produce nearly quadrupled (Olsen *et al.,* 2000). Though reports of foodborne illness outbreaks do occasionally appear, the occurrence of foodborne pathogens or fruits in India and their role in foodborne outbreaks are not thoroughly studied. According to statistics from the Integrated illness Surveillance Programme (IDSP) for the years 2011–2016, foodborne outbreaks, including acute diarrheal illness, may account for over half of all outbreaks recorded. Gram-negative bacteria are more frequently the microorganisms that can withstand the washing and sanitizing process (Casillas *et al.,* 2010). Salmonella spp., *E. coli*, and the manga bacterial population that affects food were the most common microbes causing foodborne illness and outbreaks in the United States between 1973 and 1997. It has been noted that a certain pathogen occurs. Produce combinations that cause foodborne illness outbreaks, including *E. coli* 0157: H7. It was found that Salmonella and *E. coli* 0157:H7 may live longer on parsley, for 177 and 231 days, respectively (Islam *et al.,* 2004). According to Rosset *et al.,* (2004), one of the things that might lead to the development of food-borne illnesses is temperature abuse during the delivery of food. Procedures following harvest, include cutting, washing, storing, and potential contamination sources (Wachtel and Charkowski *et al.,* 2002).

The objective of this study was to isolate and characterize the pathogen bacteria from a few common fresh fruits that are referred to as "street foods." Four fresh fruits bananas, mangoes, oranges, and apples, are

gathered from the Ayodhya market under certain conditions.

Sample Collections

Fruit samples were collected from the Devkali Market, Ayodhya City, India.

Materials and Methods

The current study was conducted at Dr Rammanohar Lohia Avadh University in Ayodhya, Uttar Pradesh, India, in the department of microbiology. All of the media used in the evaluation, including as MacConkey, Eosin Methylene Blue, and Nutrient agar. Microscope slides that are used to identify bacteria are prepared using the five bacterial stain chemicals (Crystal Violet, Iodine, Decolorization, Ethanol or Acetone, and Safranin) included in the staining kit. Devkali Market in Ayodhya City is well known for having the most daily sales volume among congested areas. Aseptic procedures were used to collect four different fruit samples, which were then stored in sterile plastic containers. The university laboratory served as the location for the examination. A sequence of successive dilutions known as serial dilution is used to lower the dense culture of cells to a concentration that is more manageable. Every dilution will lower the bacterial concentration by a certain amount (10^4 and 10^6 dilution factor of each sample were analysed). After filling 5 test tubes with 9 ml of distilled water, they were autoclaved for 15 minutes at 15 pounds and 121ºC. Test tubes were autoclaved, allowed to cool, and then the serial dilution procedure was initiated. After serial dilution of each sample, a 0. 1µ sample was applied using the spread plate method on nutrient agar, MacConkey agar, EMB agar, in that order. Using this method, the medium is made, chilled, and then added to the sterilized petri plates. The plate is then inverted and BOD is incubated for a full day at 37°C once the media has hardened. 100 ml of Nutrient Agar, 100 ml of MacConkey Agar, 100 ml of Eosin Methylene Blue Agar were produced and autoclaved at 15 pounds for 15 minutes at 121°C in order to isolate the bacteria. Following autoclaving, the media are allowed to cool. After that, sterile petri dishes are filled with them. Pour each medium into two plates. After allowing the plates to solidify, they are inverted and kept in an incubator at 37°C for a full day. Using this method, a 0.1µ sample is added to the solidified plate and distributed using a spreader. The plates were spread, then covered with parafilm and left in a BOD incubator at 37°C for 24 hours. In order to obtain pure culture colonies, the colonies that were seen after 24 hours were re-streaked from mixed colonies. Colony forming unit can be calculated by using following Formula-

$$\text{CFU/ml} = \frac{\text{Number of colonies} \times \text{Dilution Factor}}{\text{Volume of culture plate}}$$

To achieve a pure growth, the cultures from each test tube were re-inoculated onto nutrient agar. Using the pure culture on the nutrient agar plate, bacteria were identified. This technique divides bacterial species into the two major categories of Gram positive and Gram negative. By identifying peptidoglycan, Simple Staining, Negative Staining and Gram staining techniques must be used to distinguish between gram-positive and gram-negative bacteria as well as to determine the organization, size, shape, and other characteristics of each bacterial cell, which is found in the cell wall of Gram-positive bacteria, Gram staining distinguishes between bacteria based on the chemical and physical characteristics of their cell walls (Aneja, 2007; Cappuccino and Sherman *et al.,* 2005; Vinay *et al.,* 2021).

Results and Discussion

Total four samples were analysed of fresh fruits samples including Apple, Banana, Mango and Orange, where Apple at 10^4 dilution factors showed 1.50×10^4 CFU/ml at the same time 10^6 dilution factor showed 1.32×10^6 CFU/ml. Another one sample Banana at 10^4dilution factors showed 8.8×10^4 CFU/ml and at 10^6 dilution factor showed 4.0×10^6 CFU/ml. Mango sample at 10^4 dilution factor showed 1.60×10^4 CFU/ml and at 10^6 dilution factor 2.4×10^6 CFU/ml. Orange sample at 10^4 dilution factor showed 6.4×10^4 CFU/ml and at 106 dilution factor showed 5.2×10^6 CFU/ml. (Table No.2: Table of CFU).

Different staining results were suggested that Apple showed monococcus morphology with gram staining while Bacillus and coccus morphology showed with negative staining and staphylococci showed at simple staining. Banana sample showed monococcus morphology with gram staining while bacillus and coccus morphology showed with negative staining and staphylococci appeared with simple staining. Mango sample showed staphylococci with gram staining while bacillus and coccus showed with negative staining and staphylococci has been observed with simple staining.

Table.1 Sampling Site

Fruit Name	Sampling Site
Apple	Devkali Market
Banana	Devkali Market
Mango	Devkali Market
Orange	Devkali Market

Table.2 Table of CFU

S. No.	Sample	Dilution Factor	No. of Colonies	CFU/ml
1.	Apple	10^4	150	1.50×10^4
2.	Apple	10^6	132	1.32×10^6
3.	Banana	10^4	88	8.8×10^4
4.	Banana	10^6	40	4.0×10^6
5.	Mango	10^4	160	1.60×10^4
6.	Mango	10^6	24	2.4×10^6
7.	Orange	10^4	64	6.4×10^4
8.	Orange	10^6	52	5.2×10^6

Table.3 Colony Morphology

S. No.	Isolates	Colony Morphology		Staining Data		
		Shape	Colour	Gram Stain	Negative Stain	Simple Stain
1.	Apple	Short Rod	Purple	Monococcus and Bacillus	Bacillus, Coccus	Staphylococci
2.	Banana	Short Rod	Purple	Monococcus and Bacillus	Bacillus, Coccus	Staphylococci
3.	Mango	Short Rod	Purple	Staphylococci	Bacillus, Coccus	Staphylococci
4.	Orange	Short Rod	Purple	Staphylococci	Bacillus, Coccus	Staphylococci

Orange sample showed staphylococci with gram staining and with negative staining it appeared bacillus and coccus and simple staining result gave staphylococci. The aforementioned table makes it evident what may be determined about the staining data and colony shape of orange, banana, mango, and apple. We separate and identify microorganisms that are the result of contamination from fresh fruits. We can separate and count fresh fruits using both selective and non-selective media (Apple, Banana, Mango, Orange). Fresh fruits were discovered to have high levels of bacterial population (> 1000 CFU/100 ml). These findings showed that there may be a major risk to the health of the Ayodhya community. These findings thus pointed to the need for promoting sanitary standards in the purchasing and sale of fresh fruits. From the data presented current in the study, it is obvious to claim that fruits Contains bacterial count up to 1.0×10^5 cm^2 on their surfaces. Improper washing of fruits adds these bacteria to extract leading to contamination. Use of unhygienic water preservation without refrigeration unhygienic surroundings often with swarming houseflies, fruit flies and airborne dust can also act as source of contaminations. So, it is very important to educate ourselves about food safety and hygienic practices.

Author Contributions

Prashant Singh: Investigation, formal analysis, writing—original draft. Ranjan Singh: Validation, methodology,

writing—reviewing. Ajad Patel:—Formal analysis, writing—review and editing. Induja Tiwari: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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