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Nitin Kasture
Assistant Professor,
Department of Microbiology,
Siddharth College, Jafrabad,
Dist. Jalna, Maharashtra, India

Correspondence
Nitin Kasture
Assistant Professor,
Department of Microbiology,
Siddharth College, Jafrabad,
Dist. Jalna, Maharashtra, India

Bacteriological analysis of fresh vegetables, fruits from local market

Nitin Kasture

Abstract
Vegetables and fruits are highly nutritious, sources of vitamins, minerals, fibers etc. and these are part of our daily diet. Now days due to green revolution in India we are getting more productions of vegetables and fruits and in local market variety of fresh vegetables and fruits are available from the nearby villages. But main problem is associated with the quality of vegetables and fruits. Are they hygienic to consume? Because during cultivation, harvesting, transportation, handling these are get contaminated with pathogenic microorganisms which leads to severe problems to community. So, from this point of view, in current research, bacteriological analysis was performed. Total 60 samples are randomly collected from market and street vendors of Jafrabad town viz. were analyzed. Bacteriological examination of these isolates confirms contamination of fresh vegetables, fruits with pathogens like *Escherichia coli*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Proteus* are found predominant.

Keywords: Bacteriological analysis, vegetables and fruits, *Escherichia coli*, *Pseudomonas*

1. Introduction
In India, vegetables and fruits are consumed widely as an energy source for both vegetarians and non-vegetarians as they are rich in vitamins and minerals. Raw vegetables and fruits are consumed as a salad with breakfast, meals, and dinners. But, due to unhygienic conditions many pathogenic microorganisms reside over them which causes many food-borne infections, such as listeriosis, salmonellosis etc.

It has been observed that the farmers of villages are using waste water, from rivers, nalas which is mainly contaminated by coliform groups for irrigation purposes and washing of vegetables and fruits which are main source of pathogenic microorganisms in fresh fruits and vegetables [1]. Fruits and vegetables get contaminated at each step from cultivation to consumers. Many studies have been carried out on various aspects of fruits and vegetables contamination at different sources [2]. Enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns during food-related outbreaks [3]. Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables and fruits grown in or fertilized with contaminated soil or sewage [4]. It was reported that vegetables such as carrots, radishes, tomatoes, cabbage, Cucumber, coriander carries *Escherichia coli*, *Staphylococcus aureus, Enterobacter sp, Klebsiellia sp., Providencia sp, and Pseudomonas aeruginosa* [5]. The incidence of food borne outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years [6].

2. Materials and methods
2.1 Media
All the bacterial media used were procured from HiMedia i.e. Nutrient Agar, Nutrient Broth, MacConkey Agar, Bacillus cereus Agar, Bile Esculin Agar, Eosin- methylene blue agar, Triple sugar Iron agar, Mannitol salt agar etc.

2.2 Readymade Kits
Hi25TM Enterobacteriaciae Identification Kit (HiMedia, KB003), HiDtect Universal Food pathogen Identification Disc (HiMedia, DT010), Grams Staining Kit (HiMedia, K001).

2.3 Sample collection
Randomly collected 7 samples of vegetables viz. Spinach, Methi, Brinjal, Cabbage, Cauliflower, Coriander, Tomato and 5 fruits Apple, Banana, Guava, Chikoo, and Pomegranate

collected from local market and street vendors of Jafrabad town. Samples were collected in the sterile polythene zip bags and transported to laboratory for microbial analysis. These samples were collected weekly from the market. These samples were kept in the refrigerator at 4°C for later use.

2.4 Isolation of Pathogenic Bacteria:

25 gm of vegetable and fruits samples were rinsed thoroughly with sterile water and these were serially diluted. The highest three dilution were taken for analyzing the total microbial count. The amount of 0.5 ml of sample was spreaded over Nutrient agar media using sterile spreaders. The plates were incubated at 37 ºC for 18-24 hours for the appearance of colonies. Isolated colonies were sub-cultured in nutrient broth and streaked over different selective-cum-differential media agar plates i.e. MacConkey Agar, Bacillus Cereus Agar, EMB agar and Bile Esculin Agar, mannitol salt agar and were incubated at 37ºC for 18-24 hours. The pure bacterial colonies obtained were primary identified using morphological analysis. Each isolated pure culture was maintained at 4ºC for further analysis [7].

2.5 Total Plate Count of Bacteria (CFU/ml)

Microbial load in each vegetable sample was determined as CFU/ml and was calculated using formula [8]. Cfu/ml = {(No. of colonies X dilution factor) / volume of inoculums]

2.6 Identification of Microorganisms

2.6.1 Morphological identification

The isolated bacteria were identified on the basis of negative staining and Gram's staining [8].

2.6.2 Selective-cum-differential Agar media based identification

The pure isolated colonies were grown on media like Bacillus cereus agar, MacConkey agar, EMB agar, Mannitol Salt agar and were identified on the basis of characteristic growth appearance.

2.6.3 Biochemical Identification

The isolated bacterial colonies were confirmed by Biochemical kits (Universal Food pathogen Identification Disc, Hi25TM Enterobacteriaciae Identification Kit and TSI test) and the results were interpreted as per interpretation chart and identification index following kit protocol**.**

3. Results and discussion

A total of 60 samples (7 fresh vegetables and 5fresh fruits, 5 samples for each) are randomly collected from market and street vendors of Jafrabad town in sterile polythene bags. These samples then brought to laboratory under sterile conditions 25 gms of each sample was taken for dilution. After dilution, more diluted samples were used for inoculation onto various media as described earlier. After 18-24 hrs. incubation well isolated colonies were picked for Grams staining, Biochemical tests, total plate count, Coliform count and screened for the presence of pathogens. By standard methods these were identified as *Escherichia coli, Salmonella, Staphylococcus aureus* and *Pseudomonas.* Among these microorganisms the most predominant was *Escherichia coli* (63.33%), followed by *Pseudomonas* (31.66%), *Staphylococcus aureus* (30.00%), *Proteus vulgaris* (28.33%) and *Salmonella* (25.00%) as shown in Table No.1.

Table 1: Pathogenic Bactria isolated from various vegetables and fruits. (No. of Samples taken-05 each fruit and vegetable).

Sr. No.	Name of Fruits and Vegetables	Number of Pathogenic Bacteria / Samples.				
		Escherichia coli	***Salmonella vulgaris***	***Proteus***	***Staphylococcus aureus***	***Pseudomonas***
1.	Apple	4	2	2	3	3
2.	Banana	3	2	2	2	3
3.	Guava	1	-	1	1	2
4.	Chikoo	4	1	-	1	2
5.	Pomegranate	2	2	-	2	1
6.	Spinach	2	2	3	1	-
7.	Methi	4	2	1	2	-
8.	Brinjal	4	1	1	2	2
9.	Cabbage	5	1	2	1	1
10.	Cauliflower	4	-	2	1	1
11.	Coriander	3	1	2	1	2
12.	Tomato	2	1	1	1	2
	Total	38	15	17	18	19
	Percentage	63.33%	25.00 %	28.33%	30.00%	31.66%

4. Conclusion

On the basis of the present study it was concluded that four bacterial isolates i.e. *Escherichia coli*, *Pseudomonas*, *Staphylococcus aureus*, *Proteus vulgaris* and *Salmonella* were isolated and identified from the fresh vegetables and fruits. Among the above *Escherichia coli* and *Pseudomonas* were the dominant species. Bacterial contamination may be present due to improper handling, unhygienic transportation condition and improper storage. To avoid the spread of infections, practices like washing with potable water, use of disinfectants before it is consumed. It is everybody's duty to take control measures to avoid foodborne infections for the safety of community.

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