

Isolation and Identification of Pathogenic Bacteria from Fresh Fruits and Vegetables in Chittagong, Bangladesh

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Abstract Fresh fruits and vegetables are an essential part of people's diet all over the world. Since they are typically eaten raw and often without heat treatment or thorough washing, so they serve as vectors for the transmission of pathogenic micro-organisms associated with human diseases. This study aimed at isolating and identifying the pathogenic bacteria from selected popular fresh fruits and vegetables which are known as street food. A total of 7 fresh fruits and vegetables were collected from different open markets of Chittagong with aseptic condition. Selective and non-selective media were used to isolate and enumerate the bacteria. All the species were identified on the basis of morphology, biochemical tests (IMViC) and selective differential culture media. Among the samples *Vibrio* spp., *Lactobacillus* spp., *Pseudomonas* spp. and *Salmonella* spp. were dominant. All of fresh fruits and vegetables were heavily contaminated with coliform and fecal coliform (> 1100 CFU/100ml). Range of microbial count of guava was 2.1×10^4 CFU/ml, apple was 4.3×10^4 CFU/ml, tomato was 8.2×10^4 CFU/ml, cucumber was 5.0×10^4 CFU/ml, carrot was 1.3×10^4 CFU/ml, hog plum (Amra) was 7.5×10^4 CFU/ml. This results indicated that the serious concern for the people of Chittagong area as it can be a great risk for human health. So this results, suggested the necessity to create awareness about hygienic practices in selling and buying fresh fruits and vegetables.

Keywords Pathogenic bacteria, Isolation, Identification, Fruits, Vegetable

1. Introduction

In recent years, outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables have increased, despite their nutritional and health benefits [1,2]. Human disease outbreaks have been recognized as being caused by contaminated vegetable and fruits consumption, several studies have been published describing the bacterial contamination of intact vegetables and fruits in open markets [3]. Raw vegetables host a variety of pathogenic microorganisms that may be spread over the plants or occur in the plant tissues as micro colonies [2]. Some diverse factors may be affected the variation of microbial profile of vegetables including normal microflora of soil, animal manure derived flora, irrigation or sewage water, transportation and unconscious handling by retailers [Ref?]. Microbial contamination is commonly exposed to fruits and vegetables by contact with dirt, dust and water and by handling at harvest or during post-harvest processing [4,5]. Therefore, a wide range of microorganisms are present including plant and human pathogens. Due to lack of

surveillance and inadequate screening of these raw vegetables in Chittagong region, most outbreaks have become undetected and there is very little information available in the literature so far [6].

In another study, it was found that bacterial contamination of fruits and salad vegetables (tomato, cucumber etc.) was correlated with the fact that it is typically eaten without thermal treatment. A major contributing factor to contamination is use of untreated wastewater and manure as fertilizers for the production of fruits and vegetables [7]. In Bangladesh various fruits and vegetables are sold in open markets and most of them are eaten raw, which is suitable medium for bacterial contamination [8]. In developing countries like Bangladesh, both poverty and poor sanitation is common, faecal contamination of domestic and commercial food is likely to occur, and illness has been traced to the ingestion of faecally infected food in multiple outbreaks [9]. The ingestion of infected fresh vegetables and fruits has been related to many outbreaks of human gastro-enteritis [10].

The use of untreated waste water and manure as fertilizers for the cultivation of fruit and vegetables is a significant contributor to contamination [11,12].

Fresh fruits and vegetables can harbor large and diverse populations of bacteria. However, most of the research on fresh fruits and vegetables has focused on their isolation and, as a result, we know far less about the overall diversity and

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molecular characteristics of those bacterial communities [13]. We addressed these knowledge gaps by isolating bacteria from different fruits and vegetables and assessing their molecular characteristics through 16 S rRNA sequencing approach.

The present study was undertaken to isolate and identify pathogenic bacteria from fresh fruits and vegetables that are very popular in Chittagong region, Bangladesh.

2. Materials and Methods

Sample collection

A total of 7 samples of fresh fruits and vegetables were collected from two open markets (Baluchara, Masjid Market and Oxygen market) of Chittagong city. The samples were Cucumber (*Cucumis sativus*), Carrot (*Daucus carota*), Tomato (*Solanum lycopersicum*), Hog plum (*Spondias mombin*), Guava (*Psidium guajava*), Apple (*Malus domestica*) and Jujube (*Ziziphus*). All samples were collected in a sterile polythene bag in an insulated ice box to maintain temperatures ranging from 4° to 6°C and analyzed within one hour of receipt.

Preparation of samples

Within an hour of collection, the samples were taken into the laboratory and rinsed for each with 100 ml of distilled water, then diluted 10 fold serial. After washing the vegetables surface 10 ml of washed aqueous suspension of each sample was mixed with 90 ml Luria Bertani (LB) broth and incubated for 24 h at 37°C. This overnight culture in LB broth was used for the isolation and identification of strains on selective media in streak plate technique [14,15].

Isolation and identification of bacteria

The bacteria were isolated and enumerated by growing them on selective and non-selective mediums such as nutrient agar was used for total viable bacterial count (TVBC) and for total coliform (TC) and fecal coliform (FC) count MacConkey broth was used. One loop full culture from LB broth was streaked over selective media and kept it for incubation overnight at 37°C.

Eosin methylene blue (EMB) for *E. coli* and thiosulfate citrate bile sucrose agar (TCBS Agar) for *Vibrios Spp.*, *Vibrio cholera* like organism (VCLO), xylose lysine deoxycholate agar (XLD agar) and *salmonella-shigella* sgar (S-S Agar) for *Salmonella*, tomato juice agar for *Lactobacillus spp.* Cetrimide agar for *Pseudomonas spp.* and mannitol salt phenol-red agar was used for *Staphylococcus spp.*

For identification confirmation IMViC tests were done for every species [16].

Bacterial enumeration

Spread plate method was used for bacterial enumeration to determine the number of colony forming units (CFUs) [17].

Statistical Analysis

For statistical analysis, the SPSS program (V12) was used

and the T test was performed to determine the significance of the difference between groups. At 95 percent conviction ($p < 0.05$), the significance between the values was assessed [18].

3. Results

Total Viable Bacterial Count

In this study, all sampled fruits and vegetables were contaminated. The microbial load of fruit and vegetables varied with the types. Range of microbial count of guava was 2.1×10^4 CFU/ml, apple was 4.3×10^4 CFU/ml, tomato was 8.2×10^4 CFU/ml, cucumber was 5.0×10^4 CFU/ml, carrot was 1.3×10^4 CFU/ml, hog Ppum (Amra) was 7.5×10^4 CFU/ml and jujube (Boroi) was 1.2×10^4 .

Isolation and identification

A total of 4 bacteria were isolated from each of the samples and they were identified on the basis of the cultural, morphological and biochemical characteristics (Table: 1).

Yellow colonies from TCBS plates were *Vibrio cholera*, black centers on XLD were *Salmonella spp.*, opaque colonies from tomato juice agar plates were *Lactobacillus spp.*, blue-green colonies from cetrimide agar plate was *Pseudomonas spp.* (Fig: 1).

Among the samples-*Pseudomonas spp.* (50%), *Vibrio spp.* (20%), *Lactobacillus spp.* (15%), (0.37%) and *Salmonella spp.* (0.37%).

Highly presence of these bacteria is serious concern for the people of this region. Necessary step should be taken as early as possible to eliminate the presence of these bacteria for avoiding any future chance of outbreak related to food borne pathogen.

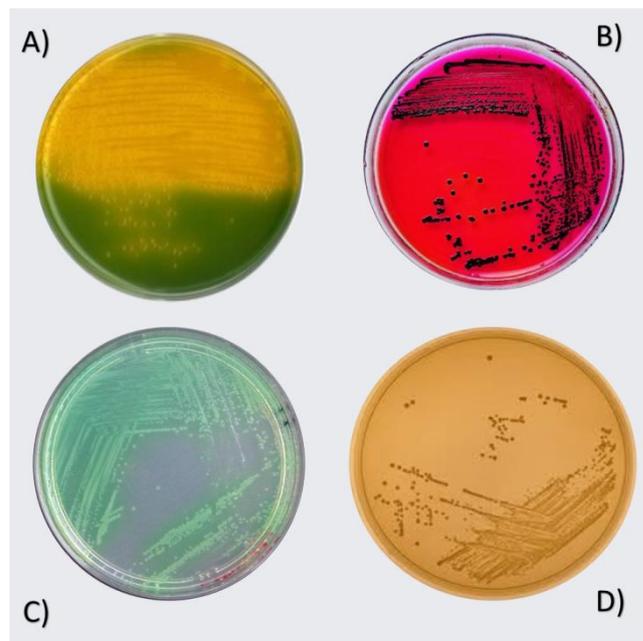


Figure 1. A) *Vibrio spp.* B) *Salmonella Spp.* C) *Lactobacillus spp.* D) *Pseudomonas spp.*

Table 1. Biochemical results

Isolate No.	Types of bacteria	Indole test	Methyl red test	Voges-Proskauer test	Citrate test
01	<i>Vibrio spp.</i>	+ve	-ve	-ve	+ve
02	<i>Lactobacillus spp.</i>	-ve	-ve	-ve	-ve
03	<i>Pseudomonas spp.</i>	-ve	-ve	-ve	+ve
04	<i>Salmonella spp.</i>	-ve	+ve	+ve	-ve

4. Discussion

During cultivation in fields or orchards or during harvesting, post-harvest handling, refining and distribution, vegetables and fruits become infected with pathogenic microorganisms. [19].

The presence of all four types of bacteria in the fruits and vegetable samples is probably a reflection of the nature of the two retail outlets in the study [14]. This result highlights the fact that fresh vegetables could be contaminated with pathogenic bacteria and thus could possibly act as a transmission vehicle of many diseases. The prevalence of coliform in guava was 2.1×10^4 CFU/ml, apple was 4.3×10^4 CFU/ml, tomato was 8.2×10^4 CFU/ml, cucumber was 5.0×10^4 CFU/ml, carrot was 1.3×10^4 CFU/ml, hog plum (Amra) was 7.5×10^4 CFU/ml and jujube (Boroi) was 1.2×10^4 which clearly indicated that the unhygienic condition of open markets.

The *Pseudomonas* spp. was present in almost all the samples where presence of *Salmonella* spp. was less in among the samples. The detection of *Vibrio cholera* in particularly 20% of the samples is a matter of concern.

Identification of *Vibrio* spp., *Lactobacillus* spp., *Pseudomonas* spp. and *Salmonella* spp. is a clear sign of serious health crisis alert for of the people living in this area.

This was clear that samples from wet markets yielded a higher proportion of bacteria because it could be seen that the way vegetables were treated on the wet markets was less hygienic. The surroundings and places for vegetable displays on markets were not clean and tidy, and the handlers did not wear gloves while the vegetables were being handled. Contamination might be occurred through a contaminated container for transporting and improper handling [20]. Apart from that, the vegetables at supermarkets sometimes have a long holding time, which could contribute to the accumulation of pathogenic bacteria [21].

5. Conclusions

This study demonstrated the alarming presence of pathogenic bacteria among the most common and popular fresh fruits and vegetable in this area. The result also indicated that the current hygiene condition of selling and buying zone of fresh fruits and vegetable in Chittagong. This study provided a general overview of the microbiological quality of fresh fruits and vegetable in Chittagong which will help to take necessary step to make sure the hygiene condition during fruits and vegetables selling and buying.

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