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# Prevalence and Antibiogram Profiling of Rotten Fruits from Different Areas of Dhaka City, Bangladesh

**Mahmuda Akhter Akhi[1], Avijit Banik[1], Oshin Ghurnee[1,2], Nantu Chandra Das[3], Showmitro Nondi[1] and Maruf Abony[1*]**

*[1]Department of Microbiology, Primeasia University, Banani, Dhaka - 1213, Bangladesh.*
*[2]School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, United Kingdom.*
*[3]Department of Microbiology, Jagannath University, Dhaka -1100, Bangladesh.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors MAA, AB and MA designed the study. Authors AB and MAA managed the experimental process and analyses of the raw data. Authors OG, AB and MA wrote the protocol and the first draft of the manuscript. Author NCD managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

Fruits are highly nutritious, sources of vitamins, minerals, fibers etc. and these are part of our daily diet. However, during cultivation, harvesting, transportation, handling fruits get contaminated with pathogenic microorganisms which leads to severe problems to community. Owning to this point, in current research, bacteriological analysis was performed on Sofeda, Pineapple, Grape, Banana, Apple, Orange, Guava, papaya, Jujube and Starfruit. Total 50 samples were randomly collected from market and street vendors of Dhaka city. Higher numbers of rotten fruits were present in wholesale markets. 35 strains were isolated which included *Salmonella* spp., *Acinetobacter* spp.,

**Corresponding author: E-mail: mabony2011@gmail.com;*

*Klebsiella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Staphylocoous aureus* and *E. coli.* They were identified using biochemical test and antibiogram for selective isolates. In case of drug resistance of isolates, majority exhibited resistance against Erythromycin, Vancomycin and Amoxycillin and showing sensitivity against Ciprofloxacin and Ceftriaxone. It was observed in the current study that 100% isolates were resistant against Erythromycin, followed by Amoxycillin 90.63% and vancomycin 86.25%, where only 35.27% isolates were resistant against Ciprofloxacin. In case of sensitivity 64.73% isolates were sensitive against Ciprofloxacin followed by Ceftriaxone 66.25%.

*Keywords: Rotten fruits; pathogen detection; antibiogram; food borne diseases; food safety.*

## 1. INTRODUCTION

Food Spoilage refers to chemical and biological change of food under any condition and degradation of quality, be it microbiological or nutritional, turning food undesirable or unacceptable for human consumption [1]. Fruit spoilage by microorganisms is such a process and is a leading cause for economical loss even with modern preservation techniques. Post-harvest decay is the main reason for spoilage of fresh fruits. A bruised fruit releases oxidizing enzymes due to chemical reaction that accelerates fruit rotting. Pectin degradation by bacteria causes tissue softening, turning the fruit texture slimy entirely or partially following to which starch and other carbohydrates are degraded turning the taste and odor unpleasant [2]. Microorganisms can transform raw foods into fermented processed foods while acting as a reservoir for disease spread thus effecting food quality and human health.

Fruits possess ideal environment to support survival and growth of microorganisms. The internal portion of fruits contains high concentration of water activity, sugars, minerals, vitamins and amino acids [3]. Spoilage means food becoming unfit to consume for human due to chemical change [4]. Spoilage causing microorganisms colonize and create lesions on healthy plant tissue [5]. Fresh fruit and vegetable consumption has increased by 25.8% and 32.6%respectively in recent years due to people's interest in living a healthy lifestyle. If this continues, the future seems promising. It can be assumed that in the next 10 years, consumption of processed fruits and vegetables would significantly decrease and total per capita consumption of fresh fruits and vegetables would rise subduing the previous [1].

During the last few decades, consumption of fruit products has significantly increased in Bangladesh by more than 11.5% on average, for their increasing cultivation and increasing awareness in healthy eating habits. In recent times, Bangladesh is one of the most significant countries to yield some notable amount of fruits such as second in cultivation of Jackfruit, seventh place in cultivation of Mangoes, eighth in Guavas in world fruit production. Java plum (jaam), litchi, jujube, star fruit (kamranga), papaya, wood apple, lemon, pineapple, watermelon, lotkon (Burmese grape), custard apple, sapodilla (safeda) and melon are some of the other fruits commonly growing in Bangladesh [6]. In Bangladesh, consumption of fruit per year is 100 kg. Daily intake of fruit and vegetable in Bangladesh in average is 68.5 grams per day which is below the required intake [7].

In 2010-2011, Bangladesh Bureau of Statistics reported that post-harvest losses occurred among 12 fruits and vegetables ranging from the lowest at 8.1% (litchi) to 32.4% (jackfruit).In Bangladesh, Banana (30.8%), Tomato (27.64%), Cabbage (27.64%) and Country bean (24.29%) were the following fruits with highest amount of spoilage previously reported. Also mango, orange, pineapple, brinjal, cucumber and cauliflower massively suffered losses [8]. In other Asia pacific countries, spoilage of fruits and vegetables have also been reported—in India it was 40%, 20-50% in Indonesia, 20-50% in Korea, 27-42% in the Philippines, 16-41% in Srilanka, 17-35% in Thailand and 20-25% in Vietnam [9].

Fresh fruits have vital parts with multi-dimensional nutritional benefits important for human diet and also the traits of natural antimicrobials and antioxidants [10-12].Conversely, fresh fruits may play a role as potential vehicles for the transmission of bacterial, parasitic and viral pathogens to the consumers ultimately leading to the possibility of onset for food borne diseases [13-16]. Such microbial contamination in fresh produces have been observed to take place from humans,

animals and environmental sources during growth, harvesting, transportation and also due to the unhygienic processing and handling of the products [10,13,14]. A major spoilage agent includes bacteria such as *Campylobacter spp., E. coli O157:H7, Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Shigella spp., Erwinia spp., Enterobacter spp., Propionibacterium cyclohexanicum, Pseudomonas spp.*, and lactic acid bacteria which are capable of causing spoilage in fruits and juices [17]. Fungi such as *Penicillium* spp., *Aspergillus* spp., *Eurotium* spp., *Alternaria* spp., *Cladosporium spp.*, *Paecilomyces* spp., and *Botrytis spp.* have exhibited involvement in the fruit spoilage [18].

The following study was carried out to investigate the microbiological status in rotten fruits collected from local vendors of different areas of Dhaka city, Bangladesh.

## 2. METHODS AND MATERIALS

### 2.1 Sample Collection

Unwashed and unprocessed rotten fruits were collected from 5 different areas around Dhaka city-- Savar, Mirpur, Uttara, Mohakhali and Banani between November 2018 to May 2019. Ten different types of fruits were collected from each location with a total of 50 in number (Table 1). Fruits sold by street vendors often do not include rotten fruits as they are periodically removed from display, which is a major cause for lack of available rotten fruit samples. All samples were collected in sterile plastic Stomacher bags from local fruit markets and local fruit vendors and brought in inside a cool ice boxes (4ºC) within 2 hours of collection. Processing and further assessment was conducted at the Department of Microbiology, Primeasia University, Dhaka, Bangladesh [19-20].

### 2.2 Sample Preparation and Isolation of Presumptive Organisms

For enrichment, 25 grams of rotten fruits was added to 225 ml of buffered peptone broth. Then samples were homogenized with 120 rpm at 37ºC for 35-48 hours. After being homogenized, 1ml of each sample were transferred to different selective enrichment media such as 1 ml of sample transferred in 9 ml Tetrathionate broth, Phenol Red lactose broth and Alkaline peptone water (APW). They were incubated overnight at 37ºC. After incubation period, enriched samples were transferred from TT broth by the four-way streak method in Salmonella-Shigella agar (SS agar) and Bismuth Sulphate Agar (BSA) for detection of *Shigella spp.* and *Salmonella spp.*. Samples from Phenol red lactose broth were transferred to Eosin Methylene Blue (EMB) agar and MacConkey agar for detection of *E. coli* and other enterobacteriacae and APW samples were transferred to Thiosulfate-Citrate-Bile salt-Sucrose (TCBS) Agar which was used for detection of *Vibrio spp*. An enriched sample from buffered peptone water was transferred to Manitol Salt agar and Cetrimide agar by streak plate method (Fig. 1). All selective Medias were incubated 24±2 hours at 37ºC. After incubation, single colonies were picked and were further sub cultured again in the selective Medias to get isolated pure colony [19].

**Table 1. Rotten fruits collected from Dhaka city areas**

| Location | Fruit samples |
|---|---|
| Savar | Sapodilla (Sofada), |
| Mirpur | Pineapple, Orange, |
| Uttara | Guava, Papaya, Jujube, |
| Mohakhali | Star fruit, Grape, Apple, |
| Banani | Banana |

### 2.3 Identification of Isolates

The bacterial isolates were identified based on their morphological characteristics, Gram stain of pure culture and biochemical tests according to the Bergey's Manual of systematic Bacteriology and database for different isolates [21-22].

### 2.4 Antibiogram Test for Isolates

Antibiogram of identified isolates were conducted for in vitro antibiotic resistance profiling using Kirby-Bauer method against commercially available antibiotics. Chloramphenicol (C30); Vancomycin (V30); Erythromycin (E15); Ciprofloxacin (CIP30); Amoxycilin (AMC10); Ceftriaxone (CRO30) - from Oxoid, UK were used for current study [23]. The action of the antibiotics allowed determining the inhibition of the pathogen to the degree proportional to the diameter of the zone of inhibition where it shows that the clear zone resulted from diffusion of the antimicrobial substance that surrounds the disc onto the agar medium. Using 5 ml of Muller-Hinton broth all pure culture of specific isolates were inoculated and then incubated overnight at 37ºC.

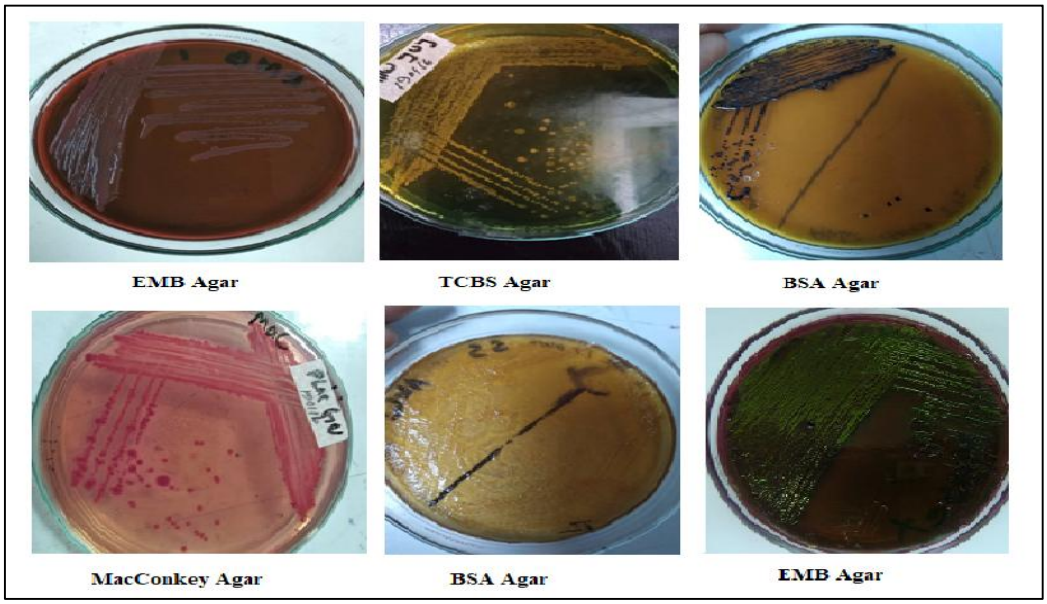


**Fig. 1. Streaking plate in different selective media (BSA Agar, EMB Agar, MacConkey Agar and TCBS Agar)**

The turbidity of the broth culture was adjusted to 0.5 McFarland standards and the standard turbid broth was spread evenly on complete surface of the plates of Muller-Hinton agar in order to measure the susceptibility of antibiotics [24].The zone diameter for individual antimicrobial agents was then interpreted into categories of susceptible, intermediate and resistant according to the guidelines from National Committee for Clinical and Laboratory Standards (NCCLS) [25].

## 3. RESULTS

In this study, 50 Fruit samples were examined of which 35 fruit samples showed the presence of bacteria and 15 samples showed other infections of fungi. Higher numbers of rotten fruits were present in wholesale markets where local markets showed lesser presence of rotten fruits due to removal of such fruits from display by the vendors [Fig. 2(a)]. In daily food intake habits, most of the fruits are consumed in their raw conditions and may lead to the onset of human diseases that is a serious threat for overall public health, as they may cause food borne illness due to transmission during post harvest [8]. During the storage period and transport, some fruits can be contaminated by other infected fruits and/or their roots [9]. Fruits that are damaged post harvest can be reservoir of microorganisms, as well as potential pathogens. Due to lack of proper handling microorganisms from rotten fruits may transmit on surface of healthy fruit, and pathogenic organisms present due to cross contamination may persist of surface of fruit. As a result when consumed raw due improper washing and cleaning food borne disease may occur [8,16]. Fruits are easily contaminated with bacterial pathogens due to cross contamination during and post harvest, which can be the major cause of bacterial infections caused by fruits. In this present study, 10 different bacterial spices were presumptive which were isolated from various rotten fruits and enumerated (Table 2).

After biochemical tests, these were identified as *Salmonella* Spp., *Acinetobacter* Spp., *Acetobacter* Spp., *Klebsiella* Spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Staphylocoous aureus* and *E.coli*. Among these microorganisms the most predominant species was *Klebsiella* Spp., (20%), followed by *E.coli* (20%), *Acinetobacter* Spp. (6%),*Acetobacter* Spp. (3%), *Vibrio parahaemolyticus* (6%), *Vibrio cholerae* (6%), *Salmonella* Spp.(11%),and *S.aureus* (28%) as shown in [Fig. 2(b)]. The biochemical tests exhibit the biochemical profiles of respective isolated genera as presented in Bergey's Manual of Determinative Bacteriology [21]. Six different Antibiotics were used against isolated microorganisms. The antibiotics showed the highest degree of resistance against Vancomycin, Erythromycin, Amoxicillin, Chloramphenicol, Ceftriaxone, and Ciprofloxacin [Fig. 2(c)].

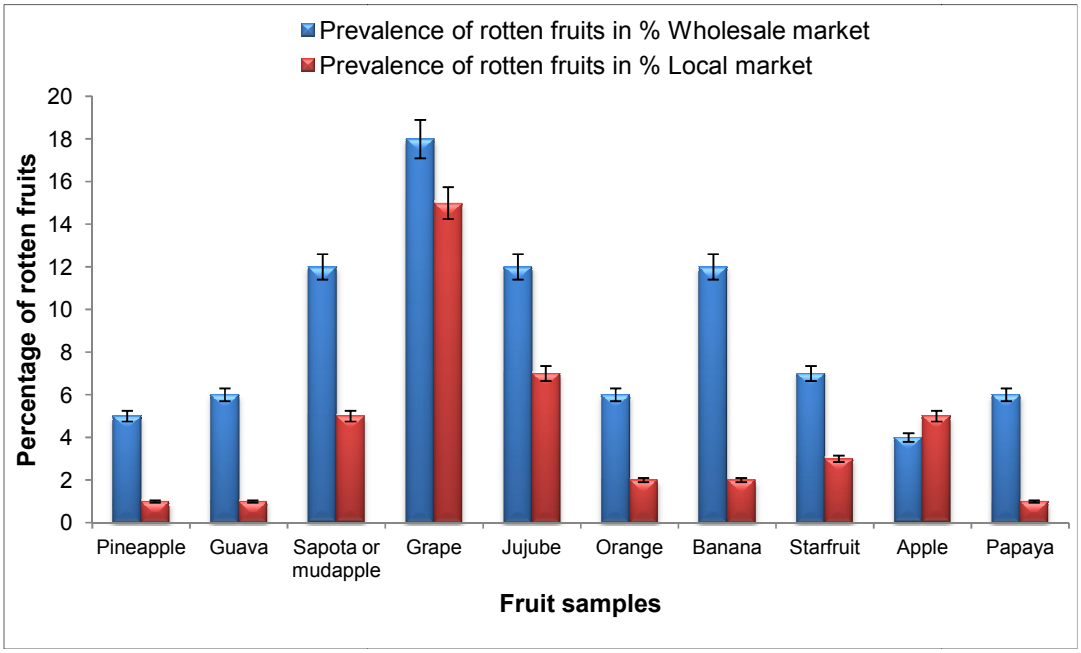


**Fig. 2(a). Prevalence of rotten fruit in wholesale and local markets**

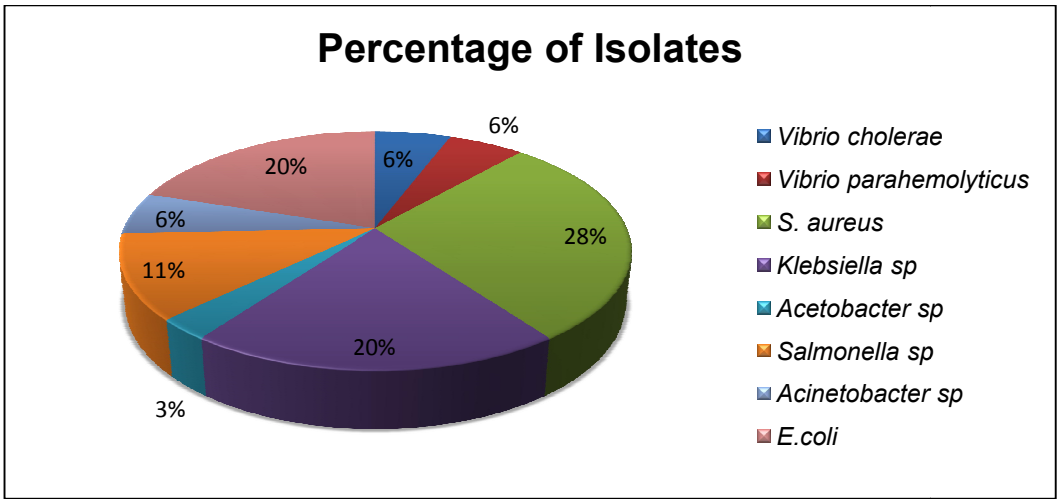


**Fig. 2 (b). Percentage of Isolated organisms from rotten fruits**

## 4. DISCUSSION

Most of the fruits are consumed in their raw states and may lead to the onset of human diseases that may put overall public health at severe risk [26]. Microorganisms are generally associated in a number of ways with the different types of fresh foods, affecting their gross quality and threatening human health hygiene [27]. Fruits are contaminated easily with bacterial pathogens by the principle of dissemination of bacterial infection. One single infected citrus fruit can be the root of infection to other fruits during storage and during transport [26].

From this study of selective fruit samples, 35 isolates showed presence of *Salmonella* spp., *Acinetobacter* spp., *Klebsiella* spp., *V. cholerae*, *V. parahaemolyticus*, *S. aureus* and *E. coli.* These were identified using tests of Identax database for bacterial identification. Out of these isolates *E. co*li, *Vibrio* Spp., *Salmonella* Spp., & *Klebsiella* Spp. possess serious threat to public health and cause food borne illness. Previous

research studies conducted in 2017 have reported similar results, where from the rotten fruit samples such as apple, banana and guava (infected from others fruits) organisms like *E. coli*, *Salmonella* and *S .aureus* were isolated [28]. In our study, *Klebsiella* spp. was also found in apple, orange, guava, pineapple as same as previous reports [28-30]. Primarily, *Staphylococcus* spp. and *Klebsiella* spp. were isolated from 30 spoiled fruit samples.

Similar results have also been reported that showed number of isolates alike to our study [29]. Majority of the isolates were similar to the findings of research conducted previously in which, *E. coli* was found in 3 different juice samples (orange, pomegranate, grape), *Staphylococcus* Spp. was found in 4 different samples (mango, orange, litchi), *Klebsiell*a spp. in 2 different samples (jujube and litchi), *Salmonella* spp. in jujube juice sample, also *Acinetobacter* spp. and *Acetobacter* spp. has been identified from same juice samples [31]. In case of drug resistance of isolates, majority of the isolated species exhibited resistance against Erythromycin, Vancomycin and Amoxicillin; and also these isolates were sensitive against Ciprofloxacin and Ceftriaxone.

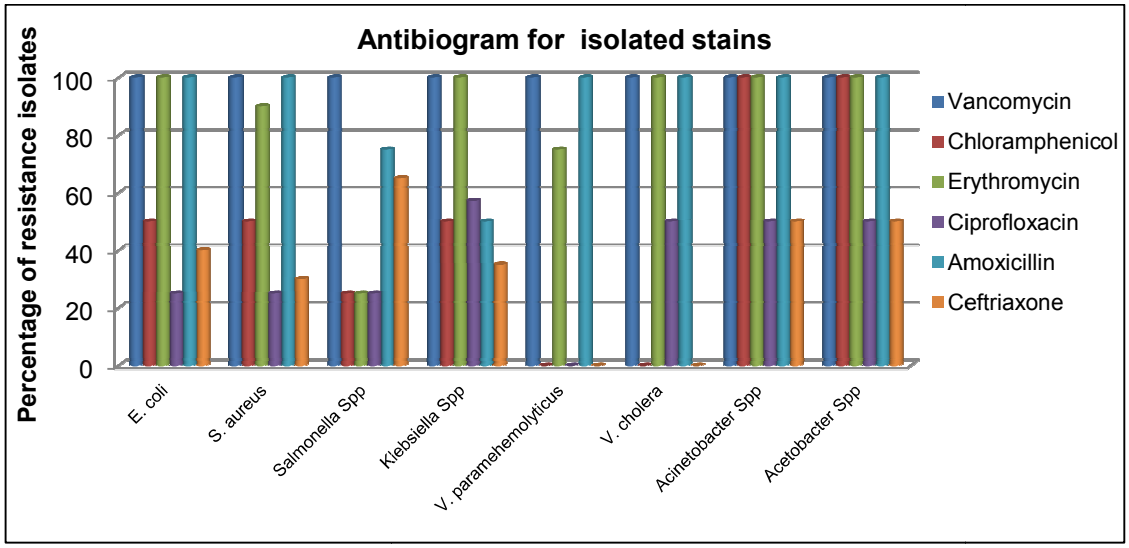


**Fig. 2(c). Occurrence of drug-resistant isolates from rotten fruits in Dhaka, Bangladesh**

**Table 2. Morphological characteristics of bacteria isolated from ten types of rotten fruits**

| Sl no | Using media plate | Colonial morphology | | | | Gram reaction | Cell morphology |
|---|---|---|---|---|---|---|---|
| | | Pigment | Consistency | Margin | Elevation | | |
| 01 | SS agar | Colorless | Plaster | Circular | Flat | - | Cocci, Bacilli |
| 02 | SS agar | Pink | Plaster | Circular | Raised | - | Bacilli |
| 03 | BSA agar | Black | Plaster | Circular | Raised | - | Bacilli |
| 04 | BSA agar | Brown | Plaster | Irregular | Raised | - | Bacilli |
| 05 | EMB agar | Green Metallic sheen | plaster | Circular | Raised | - | Bacilli |
| 06 | EMB agar | Purplish | Mucoid | Irregular | raised | - | Bacilli |
| 07 | Mac agar | Pink | Mucoid | Irregular | Flat | - | Bacilli |
| 08 | TCBS agar | Blue Green Center | Mucoid | Circular | Raised | - | Curved Bacilli |
| 09 | TCBS agar | Yellow | plaster | Circular | Raised | - | Comma Shaped |
| 10 | MSA agar | Golden Yellow | Plaster | Circular | Raised | + | Cocci |

Most of the organisms showed resistance to almost every antibiotic. Some organisms showed resistance to one or two antibiotics, such as *E. coli* and *S. aureus* showed 100% resistance to Vancomycin, Erythromycin, Amoxicillin; *Acetobacter* Spp. and *Acinetobacter* Spp. both showed resistance against Chloramphenicol. More sensitivity was observed against Ceftriaxone by the isolates--*E. coli* (40%), *Salmonella* Spp.(65%), *S. aureus* (30%) and *Klebsiella* Spp. (35%). These results were more similar compared with the previous data found of Ceftriaxone [32]. Identified *Vibrio* Spp. isolates showed 100% sensitivity against Chloramphenicol and Ceftriaxone. Our antibiotics resistance pattern was found similar to that of previous reports on antibiogram of infected routine fruit samples [33].

Although rotten fruits are not consumed directly, but in many industry and preparation of beverage items derived from fruits, such as street vended fruit juice. This in turn shows presence of various microorganisms in prepared food product from fruit [34]. In current study, it was observed that many bacterial strains isolated from rotten fruits are similar to isolates found in street vended fruit juices [34].

## 5. CONCLUSION

In conclusion it can be said that current study shows a complete bacteriological profiling of rotten fruits from local markets of Dhaka city, which is of interest for public health significance and awareness of food borne pathogens. There are many sources of contamination caused by improper handling of products such as poor water quality, transmission from badly sanitized containers and people casually carrying produce during processing, packaging, ineffective hand washing procedures, improper storage temperature and transportation. To avoid the expansion of infections, particular practices of food consumption and washing fruits before it's consumed should be considered. The government involvement for awareness on the issue should be implemented along with corrective actions and for the protection of the consumer; it is required to assess the quality of food. Food and Drug Authority could implement the food safety law more effectively by monitoring the retailers not to sell these items.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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