Microbiological Quality of Market Fruits and Their Handling Implication in Relation to Public Health


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ABSTRACT

The research study was conducted on the market fruits from selected local markets of Mymensingh, Bangladesh. The objectives of the study were to assess the microbiological quality of market fruits, hand washing and used water of hotels and chatpoti shops and to predict the comparative public health implication associated with the socioeconomic status of the inhabitants consuming the fruits and water. In fruit samples, the total viable count ranged from log 2.90 to log 6.4. No coliform bacteria were detected in fruit samples. Statistical analysis showed significant variations in microbial load in different fruits, hand washings and used water. Present study further demonstrated that there was lack of proper hygienic and sanitation measures in most of the markets, especially in town markets. Because of observed low sanitary quality, the urban market fruits should be handled and used with special attention to reduce the risk of contamination with potential pathogenic bacteria.

Keywords: Market Fruits, Microbial Quality, Hygienic Condition, TVC, TCC

Introduction

Fresh fruits and vegetables promote good health but harbor a wide range of microbial contaminations (Eni et al., 2010). Increasing health awareness has led to an increased consumption of minimally processed fruits in recent years (Warriner et al., 2009), as these do not require elaborate preparations (Amoah et al., 2009). Microbial spoilage and contaminating pathogens pose a serious problem in food safety (Abadian, et al., 2008; Warriner et al., 2009). Fruits from sources like super markets may be protected from contamination and spoilage during subsequent handling, packaging, transport and storage (Park et al., 2012). Zweiertering (2002) stated that four categories of Microbiological quality have been assigned based on standard plate counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous. Satisfactory results indicate good microbiological quality. No action required. Marginal results are borderline in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the handling of the fruits. Unsatisfactory results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices. Potentially hazardous, the levels in this range may cause food borne illness and immediate remedial action should be initiated. Actions consideration should be given to the withdrawal of suspected fruits still available for sale or distribution and recall of marketed fruits.

In Bangladesh, sellers as well as consumers are not well aware of the hygienic management in all steps of fruit production, processing, transportation, marketing and consumption (Rahman et al., 2011). Report on water used for the washing of fresh tomatoes in major markets in south-eastern Nigeria has been shown to possess a high level of microbial contamination (Ofor et al. (2009). Present study was undertaken to determine the bacterial load of fruits sold in retail market. We also aim to identify any potential pathogenic bacterial contamination and assess the situation in terms of public health hazard.

Materials and Methods

The study was conducted in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh, during the period from January to May, 2008.

Sample collection and transportation

Samples were collected from selected fruit markets of Mymensingh sadar upazila, following the methods described elsewhere (Andrews and Hammock, 2003). The fruit markets were KR market of BAU campus, Sheshmoor, Kewathkali, Shambhugonj Bridge Bus Terminal and Ganginapar of Mymensingh. A total of 20 fruits (5 apples, 5 grapes, 5 tomatoes and 5 plum), 10 water samples (used water in hotel and chatpoti shop) and 5 samples of hand washing (from various fruit sellers) were collected from different markets.

Preparation of culture media

All the culture media [Nutrient Agar (NA), Blood Agar (BA), Eosin Methylene Blue (EMB), MacConkey’s Agar (MA) and Salmonella and Shigella (SS) agar media] were prepared.
following the procedure described by BAM (2001) and Cheesbrough (2006).

### Processing of samples

Proper care was taken during the sampling procedure to prevent contamination of the samples as per procedure described by Andrews and Hammock (2003). The fruit samples were washed with distilled water. The washed water was transferred into a separate tube containing 0.1% peptone solution. Used water samples including the hand washing water were directly inoculated (0.5ml) into 4.5 ml 0.1% sterile peptone solution. The samples were then subjected to microbial assessment and analysis.

### Isolation of different bacteria from samples

After collection of samples, 100 microliters of the processed sample were inoculated onto NA media and EMB agar media by spread plate technique. The inoculated media were incubated at 37°C for overnight in an incubator. Different types of bacterial colonies were counted and isolated in pure cultures.

### Determination of total viable count (TVC)

For the determination of total viable counts, 0.1 ml of each ten fold dilution was transferred and spread on plate count (PC) agar using a sterile pipette for each diluent. The diluted samples were spread as quickly as possible on the surfaces of the plate with a sterile glass spreader. The plates were then kept in incubator at 37°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. Total viable count was expressed in a particular dilution was multiplied by the dilution factor to obtain the total viable count. Total viable count was expressed in log (CFU/ml) of samples (Cheesbrough, 2006; Garlighright, 2001).

### Determination of total coliform count (TCC)

For the determination of total coliform count the TVC method was employed. For TCC method MacConkey agar media and broths were used for bacterial propagation (Feng et al., 2002).

### Gram staining and motility test

Gram staining and Motility test using hanging drop method were employed to provide basic information about the Gram staining and Motility test using hanging drop method broths were used for bacterial propagation (Feng et al., 2002). For MR-VP tests, the tubes containing 2 ml of MR-VP broth were inoculated with the isolated organism. Tubes for VP test were inoculated at 37°C for 24 hours but for 5 days at the same temperature for MR test. To perform VP test 1.2 ml of 5% alkaloid alpha-naphthol and 0.2 ml of 40% KOH were added in each incubated VP test tube. The ingredients were mixed thoroughly and kept still. The appearance of pink color indicates its positive reaction for the presence of acetyl methyl-carbinol. The organism, isolated was found negative for VP test as the broth of E. coli culture produced yellow color when treated with the VP reagents. For MR test two to four drops of MR solution were poured in each tube. The appearance of red color was regarded positive and yellow color negative for MR test.

### Maintenance of stock culture

For the maintenance of stock culture, 1ml of 80% sterilized glycerol was used in which 1 ml of nutrient broth (NB) culture was mixed and stored at 20°C.

### Results

#### Total viable count (TVC) and total coliform count (TCC) in different samples

The TVCs of the apple washings from different market palaces are presented in Table 1. The mean±SD value of total viable count was log 2.96±0.22. The highest bacterial density was found log 3.14 at Shambhughonj area and a significantly (P<0.01) lowest bacterial density at KR market (log 2.90). In KR market there was a good hygienic condition and in other areas the hygienic condition was moderate. There was a great effect of fruits hygienic condition on the total viable bacterial count. The mean TVC were log 2.97, log 4.49 and log 6.35 for apple, tomato and plum, respectively. On the other hand, TVC in grapes were nil. This may be due to the sanitary, handling and preservation of grapes.

The bacterial density of tomato washing in different sample was significant at 1% level of probability (Table 1). The tomato of Sheshmoor with lower hygienic condition was evidenced consistently high (log 5.04), followed by Kewatkhali and Shambhughonj area (log 4.38 and 4.37 respectively). On the other hand, the lower bacterial load was observed at KR market area (log 4.34). Plum washing varied significantly (P<0.01) and are presented in Table 1. The mean±SD value of total viable count (TVC) was log 6.36±0.052. The bacterial load was highest (log 6.42) at Kewatkhali and the lowest bacterial density was log 6.29 at KR market.

In case of hotel used water the highest TVC was found in Sheshmoor (log 6.26) and lowest was KR market (log 6.14). On the other hand used water in chatpoti shop the highest TVC was found in town (log 5.94) and lowest in BAU campus (log 5.83). In this study, TCC was nil in fruits (apple, grapes, tomato, plum). This may be due to practice of good hygiene and sanitation. In this study, TCC were found in hand washing of all experimental area which was higher (log 3.20) in Shambhughonj and was lower (log 1.70) in KR market. Coliform was also detected in used water of chatpoti shop may be reasons of step from the production to consumption which may affect the microbial load.

### Isolation of different organisms in different media

#### Gram's staining and motility test

In the present study, on NB inoculation, all the organisms produced turbidity after 24 hrs incubation at 37°C. *E. coli* produced colorless colonies with hemolysis on BA, bright pink or red colonies on MC agar, greenish-black colonies with metallic sheen on EMB agar and slight pinkish colony on SS agar. In Gram’s staining under microscope the organism was smear with a sterile wooden stick or glass rod and was immersed into the H2O2 solution. If bubbles of oxygen are released, the organisms are considered catalase producers.
Table 1. TVC and TCC of different fruits and their handling implications.

<table>
<thead>
<tr>
<th>Sources</th>
<th>Apple</th>
<th>Tomato</th>
<th>Pulm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hygiene status</td>
<td>TVC</td>
<td>TCC</td>
</tr>
<tr>
<td>KR market</td>
<td>Good</td>
<td>2.60</td>
<td>Nil</td>
</tr>
<tr>
<td>Sheshmoor</td>
<td>Lower</td>
<td>3.08</td>
<td>Nil</td>
</tr>
<tr>
<td>Kewatkhali</td>
<td>Lower</td>
<td>3.11</td>
<td>Nil</td>
</tr>
<tr>
<td>Shambhugonj</td>
<td>Lower</td>
<td>3.14</td>
<td>Nil</td>
</tr>
<tr>
<td>Ganginapar</td>
<td>Moderate</td>
<td>2.90</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.96 ± 0.22**</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>0.105</td>
<td>LSD</td>
</tr>
</tbody>
</table>

Table 2. Hygienic condition, TVC and TCC per ml of hand washing water from different fruit sellers and used water from various hotels and chatpoti shops

<table>
<thead>
<tr>
<th>Sources Points</th>
<th>Hand washing water</th>
<th>Used water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hotel</td>
<td>Chatpoti shop</td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>Hygiene status</td>
<td>Good Modera... Lower Lower Lower Good Modera... Lower Good Lower</td>
<td></td>
</tr>
<tr>
<td>TVC</td>
<td>4.83 4.98 5.32 6.18 5.90 6.14 6.19 6.26 5.83 5.94</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>5.44±0.58**, LSD= 0.715</td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>1.70 2.80 2.60 3.20 3.00 Nil Nil Nil 3.30 3.70</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>2.66±0.58*, LSD= 1.547</td>
<td></td>
</tr>
</tbody>
</table>

All counts are expressed in logarithm** = P<0.01 NS = Non-significant, 1=KR market, 2=Sheshmoor, 3= Kewatkhali, 4=Shambhugonj, 5=ganginapar, 6=KR market, 7=Jabber’s moor, 8=Sheshmoor, 9=BAU campus, 10=Town

Table 3. The results of biochemical tests of different organism

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Fermentation properties with five basic sugar</th>
<th>Catalase</th>
<th>MR test</th>
<th>VP test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DX = Dextrose, ML = Maltose, L = Lactose, S = Sugar, MN = Mannitol, AG = Acid and gas, A = Acid, MR= Methyl-Red and VP = Voges-Proskauer</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Frequency distribution and percentage (%) distribution of bacteria contaminating fruit samples, hand washings and used water from various hotels and chatpoti shops

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Bacteria isolated</th>
<th>No. of sample</th>
<th>Percentage</th>
<th>Chi square test (χ²)</th>
<th>Percentage (%) distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Staphylococcus spp.</td>
<td>25</td>
<td>92%</td>
<td>0.261 NS</td>
<td>53.48</td>
</tr>
<tr>
<td>02</td>
<td>Escherichia spp.</td>
<td>20</td>
<td>25%</td>
<td>11.63</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>Bacillus spp.</td>
<td>20</td>
<td>50%</td>
<td>23.26</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>Non specific</td>
<td>25</td>
<td>20%</td>
<td>11.63</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>43</td>
<td>47.77%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant
revealed Gram-negative, pink colour, small rod shaped arranged as single or paired. On NA media, *Staphylococcus* spp showed smooth circular, opaque often yellow-pigmented colony about 1 mm in diameter after 24 hrs at 37°C. Smears from pure cultures revealed Gram positive, round shaped bacteria arranged in cluster form. *Bacillus* spp. produced haemolytic zone on BA. In Gram’s staining under microscope the organisms were shown Gram positive, rod shaped cells, arranged in chain form.

**Distribution of selected bacteria present in different samples**
Most of the samples contained the *Staphylococci* (about 92%) organisms. *Bacillus* spp. (50%) ranks the second position and *E. coli* (25%), the third position. In this study, non-specific organisms (20%) were also found all of the experimental samples. The chi square test found to be non-significant (P>0.05) which shown in Table 4. A total of 43 bacterial isolates were recovered from different fruits samples. Out of isolated organism the ranking percentage was as follows; *Staphylococcus* spp. 53.48%, *Escherichia* spp. 11.63%, *Bacillus* spp. 23.26% and non specific 11.63%.

**Discussion**

**Total viable count (TVC) and total coliform count (TCC) in different samples**
In the present study, TVC of bacteria in apple from Sheshmoor, Kewatkhalii and Gongnapar, was moderate along with a lower to moderate fruit hygienic conditions. In all of the areas, TCC was nil and the hygienic conditions were good or lower moderate. Current data are in close agreement with the findings of Stopforth et al. (2004) which was log 3.0 in apple washing water. Rahman et al., (2011) reported on some commercially packed and fresh fruit juice from Dhaka city, Bangladesh and found that the TVC varied from 10^2 to 10^5 cfu/ml – the highest was 2.4 x 10^5 cfu/ml. The also detected a large number of *Staphylococcus* and *Bacillus* spp in some samples. The author also reported that total coliforms and fecal coliforms were found in six and five (out of fifteen) samples, respectively. Among total coliforms, *Klebsiella, Enterobacter* and *E. coli* were detected.

In this study, TCC was nil in fruits (apple, grapes, tomato and pul). This may be due to practice of good hygiene and sanitation. Though, Nkere et al. (2011) stated that the level of coliform contamination in the food samples from vendors and restaurants (geometric mean count 7.64-9.21; MPN > or = 50) were above the accepted 10^2 cfu/g or MPN < or = 10 limits. Orozco et al. (2008) found total coliform count <05 log CFU per tomato. Besides this Mukherjee et al., (2004) isolated 1.5 and 9.7 percent coliform from organic lettuce and organic green pepper respectively. Tsado et al. (2013) found bacterial load of 6.1 x 10^5 cfu/g with tomatoes of rural farms and rural markets whereas 4.5x 10^5 cfu/ml with tomatoes of urban farms and markets.

In this study, TCC were found in hand washings of all experimental area - the highest (log 3.20) was from Shambhugoni and the lowest (log 1.70) was from KR market. Coliform was also found in used water of chatpoti shop. Shayo et al., (2007) found the TVC ranged from 2.75 x 10^3 to 5.4 x 10^3 c.f.u/ml in used water in Tanzania which indicate poor quality in used water. Kroger and Noll (1969) found high proportion of *E. coli* and other coliform from washed water. On the contrary, Dragan and Tratnik (1975) found 2.3% *E. coli* from used water. Delaquis et al., 2007 isolate *E. coli* from Japanese restaurant, fast food restaurant and western food restaurant in different cities of Taiwan.

**Isolation of different organisms in different media**
In the present study, the morphology, staining, cultural and biochemical characteristics, showed by different bacteria, are almost similar with the reports of BAM (2001) and Cheesbrough (2006). On the basis of these findings, the bacteria were identified as *Staphylococcus*, *Bacillus* and *E. coli*. Some non-specific bacteria were also found in the present study.

In the present study, most of the samples contained the *Staphylococci* (about 92%) organisms. *Bacillus* spp. (50%) ranks the second position and *E. coli* (25%), the third position. In this study, non-specific organism (20%) was also found all of the experimental samples. The chi square test found to be non-significant (P<0.05) which shown in Table 4. Eni et al. (2010) identified nine bacteria belonging to eight genera from 15 samples of different fruits and vegetables in Sango-Ota, Nigeria and stated that *Staphylococcus aureus* (29.2%) was the most frequently isolated followed by *Staphylococcus spp* (12.5%), *Klebsiella spp* (12.5%) and *Salmonella spp* (12.5%). *Actinomyces* (4.2%) and *Escherichia coli* (4.2%) were the least frequently isolated. Mukherjee et al., (2004) conducted microbiological analyses of fresh fruits and vegetables produced by organic and conventional farmers to determine the coliform count and the prevalence of *Escherichia coli* and *Salmonella* spp. Ameyapoh et al. (2007) found *Bacillus* spp involved in spoilage of tomato. Arthur et al. (2007) isolated *Salmonella* from fresh market tomatoes with no samples yielding *Shigella* or verotoxigenic *E. coli*.

Among the 43 bacterial isolates recovered from different fruits samples, the ranking percentage was *Staphylococcus* spp. 53.48%, *Escherichia* spp. 11.63%, *Bacillus* spp. 23.26% and non specific 11.63%. Nkere et al. (2011) found *E. coli* and *Klebsiella pneumoniae* were the two major coliforms identified among 98 coliform isolates obtained from the various food samples, of which 78 (79.6%) were assumed to be of human origin on account of their ability to grow at 44°C.

**Conclusion**

From the present study, it may be concluded that the presence of microorganisms in fruits and used water from hotel and chatpoti shop has public health importance because it might play a role for the commencement of various enteric diseases in man. The results of the study, therefore, demands the stringent supervision and implementation of food-safety practices and regular education on food and personal hygiene among food vendors.

**References**


Dragas AZ and M Tratnik, 1975. On the value of examination of drinking water and swimming pools for the presence of enteropathogenic E. coli. Microbial Abst. 10(9): 10878.


