Microbiological analysis of different categories of food items in Dhaka city, Bangladesh

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Abstract
Pathogenic microorganisms have been so far reported to contaminate a wide range of foods triggering food borne infections or intoxications including the enteric complications, abdominal pain, fever, bloodstream infection etc. The current study was attempted to observe the microbiological quality of some popular foods collected from different places of Dhaka city, Bangladesh. Conventional cultural, microscopic and biochemical tests were followed for the detection and enumeration of bacterial isolates associated with these food samples. The investigation encompassed detection of total viable bacterial count (TVBC) and presumptive identification of other pathogenic bacteria from these samples. Higher counts of TVBC, coliform, Staphylococcus spp. and fungal load were recorded as $1.46 \times 10^7$ CFU/g (yogurt 1), $4.5 \times 10^6$ CFU/g (yogurt 1), $5.6 \times 10^5$ CFU/g (raw meat) and $2.9 \times 10^3$ CFU/g (sea fish), respectively. Fecal coliform was detected only in one out of ten samples. On the contrary Salmonella spp., Vibrio spp., Shigella spp. and Pseudomonas spp. were completely absent. The antibiogram study showed that all the isolates were sensitive against Kanamycin and Azithromycin. Better sensitivity was also observed against Gentamycin and Ciprofloxacin. Varying degree of antibiotic resistance was also detected against Cefixime, Amikacin and Neomycin. Our study emphasizes the need for continuous monitoring of the various categories of food samples for the safety of public health.

1. Introduction
Food is the basic and utmost need for people and animals to survive. Sources of foods are traditionally from animals and plants. It is consumed by living beings for the constant supply of energy (Forbes, 2007). Foods are generally contaminated with various bacterial species and fungi during harvesting; processing; and through the use of associated machines, utensils, water, etc. (Frazier and Westhoff, 2007; Alam et al., 2015). The analysis of food products for the presence of pathogenic microorganisms is one of the fundamental procedures to maintain the safety and quality of food. Though a number of advancement have been observed recently in the field of food technology, foodborne diseases are still considered as a major concern for human health, in developing as well as in developed countries. Everybody, especially very young or old and immunologically weak people are vulnerable to foodborne diseases and some severe complications can happen as a result of infection (Sherin et al., 2015). Foodborne illnesses are occurred due to the action of microorganisms and/or their toxins, fungi with their related toxins, and chemical contaminants (Khamis and Hafez, 2011). The World Health Organization (WHO) listed some hazards like potentially harmful bacteria, viruses, toxins, parasites and chemicals that may be found in food. According to the WHO, one person out of 10 people becomes ill and 420,000 people die every year as a result of consuming contaminated food (World Health Organization (WHO), 2018). The International Commission on Microbiological Specifications for Food (ICMSF), 2005 brought in the concept of Food Safety Objectives (FSOs) as the maximum frequency and/or concentration of a microbial hazard (microorganisms or toxins) in a food measured tolerable for consumer safety.

Another current health related alarming issues is the threat and existence of multidrug resistant pathogens in foods. Antibiotic resistance is a feature of a microorganism (CDC, 2018). The WHO defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection caused by those microorganisms (WHO, 2014).
Antibiotic resistance is a global issue and the origin of resistance is often found in the world’s tropical or hottest regions (Collignon et al., 2018). As per the antibiotic resistance report 2019, more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result (Edwards et al., 2019).

Along these lines of considerations, the current study focused on the presumptive detection of some common food borne pathogens from the daily consumable food samples (fish, meat, yoghurt, milk, ice cream, juice). The antibiogram profile of the suspected bacterial isolates from the food samples was also tested in this study.

2. Materials and methods

2.1 Sample collection and processing

A total of eighteen food samples of various categories such as fish, meat, yoghurt, ice cream, milk and juice samples were collected from some popular super shops located in various areas of Dhaka city such as Mouchak, Shantinagar, and Badda areas. Collection of samples was done in different time intervals and taken to the laboratory as quickly as possible according to the method suggested by FDA, 2013. Before calculating the bacterial and fungal load, samples were subjected to serial dilutions up to 10⁴.

2.2 Microbiological analyses

For the estimation of total viable bacterial count (TVBC), 0.1 mL sample of each dilution was introduced onto the nutrient agar (NA) plates following spread plate technique. The nutrient agar plates were kept for incubation at 37°C for 24 hrs. For the detection of specific bacteria, from 10⁻² and 10⁻¹ dilutions of each sample, 0.1 mL of suspension was spread onto MacConkey agar, Mannitol salt agar (MSA) and Pseudomonas agar media for the enumeration of Coliform, Staphylococcus spp. and Pseudomonas spp., respectively (Cappuccino and Sherman, 2014; Afroz et al., 2015; Uddin, 2018). All the plates were incubated at 37°C for 24 hrs. The characteristic pink colonies on MacConkey agar, yellow colonies on MSA and colonies with greenish fluorescent pigmentation on Pseudomonas agar was indicative for the presumptive isolation of coliform, Staphylococcus spp. and Pseudomonas spp., respectively.

2.3 Enrichment procedure.

For the identification of Salmonella spp. and Vibrio spp., enrichment culture technique was followed. In this procedure, 1 mL of the sample was inoculated into 9 mL of selenite cystein broth (SCB) and alkaline peptone water (APW) and incubated at 37°C for 6 hrs. Following incubation, the samples were diluted up to 10⁻³ and then 0.1 mL of samples from 10⁻² and 10⁻³ dilutions were spread over Salmonella-Shigella agar (SS) and Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS) from the respective enrichment media (Cappuccino and Sherman, 2014; Tabassum and Uddin, 2016). The appearance of small blackish colonies after incubation for 24 hrs at 37°C was an indication of the presence of Salmonella spp., while the large (2-41 mm) and slightly flattened, yellow colonies on the TCBS agar referred to the presence of Vibrio spp.

2.4 Biochemical tests for the confirmative identification

Biochemical tests Identification of the isolates was carried out by major biochemical tests, for example-Triple Sugar Iron (TSI), Motility Indole Urease (MIU), Methyl-Red (MR), Voges-Proskauer (VP) and Citrate Utilization were performed following the standard methods (Cappuccino and Sherman, 2014).

2.5 Study of antibiogram

After the isolation of the bacterial isolates, antibiotic susceptibility assay was carried out against different groups of antibiotics in vitro by the Kirby-Bauer method (Bauer et al., 1966). Drug resistance was observed against Neomycin (30 µg), Cefixime (5 µg), Kanamycin (30 µg), Gentamycin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), and Azithromycin (25 µg). From overnight culture plate, a small portion of a fresh colony was transferred to Muller-Hinton broth and incubated at 37°C for 4 to 5 hrs until the growth reached to the equivalent turbidity standard of McFarland (0.5 standards). Muller-Hinton agar plates were seeded properly by spreading the inoculate using sterile cotton swab. Discs (OXOID, UK) were placed gently at a proportionate distance from each other using a sterile needle. The plates were then incubated overnight at 37°C and zones of inhibition (if any) were measured and interpreted as susceptible, intermediate and resistant categories by referring the recommended interpretative standards (CLSI, 2006).

3. Results and discussion

Foodborne diseases are the leading global problem causing considerable morbidity and mortality each year (Hanson et al., 2012). The most frequent known causes of foodborne diseases are pathogenic bacteria. In this study, the goal was to analyze the microbiological quality of different popular food items consumed by the people of Dhaka metropolitan, Bangladesh. Therefore, the current study attempted to check the presence of microorganisms in such categories food items in terms of total viable count as well as in the finding of different...
pathogenic organisms such as Vibrio spp., Salmonella spp. and Shigella spp. along with the drug resistance properties of the assumed bacterial isolates.

3.1 Isolation and enumeration of microorganisms

3.1.1 Total viable bacterial count (TVBC)

In this study, the total viable bacterial counts ranged from $2.0 \times 10^3$ CFU/g to $1.64 \times 10^7$ CFU/g which was found in commercial juice-4 and yoghurt-1 samples respectively (Table 1). This finding was quite similar to the study done by Oranusi et al. (2013) and Uddin, 2018. According to FDA guideline (2013) the acceptable limit is $5 \times 10^4$–$10^5$ CFU/g in case of total viable bacteria. So, 14 out of 18 studied samples were very much within the acceptable range as per FDA guideline.

3.1.2 Coliform, fecal coliform, Salmonella spp. and Shigella spp. count

Coliform was detected in eight out of eighteen samples of which the highest count as $4.5 \times 10^6$ CFU/g in yoghurt-1 whereas the lowest count was $1.1 \times 10^2$ CFU/g in ice cream-1 (Table 1). In this study, the detection of coliform bacteria, especially E. coli and Klebsiella spp. in these samples showed the possibility of the presence of fecally contaminated microorganisms as also suggested by Adams and Moss (2008). The lack of hygiene of the food handlers may be a parameter for the possible presence of coliforms in food items. In this study, fecal coliform was noticed in a sea fish sample. Fortunately, Salmonella spp., Shigella spp., Vibrio spp. and Pseudomonas spp. were totally absent in the selected food items. But this result was contradictory to the findings of Tambekar et al. (2008) who identified these organisms in some ready to eat street vendor food items.

3.1.3 Total Staphylococcal count

Staphylococcus spp. count was observed within the range from $1.1 \times 10^2$ CFU/g – $4.5 \times 10^6$ CFU/g which was found in ice cream-1 and yoghurt sample-1 respectively (Table 1). The existence of S. aureus in food is a sign of poor hygiene practices. S. aureus in food is connected with cross contamination occurring during processing and storage or through the contamination of raw ingredients (Akindele and Ibrahim, 2016).

3.1.4 Total fungal load

Fungus presence is an indication of the availability of various Mycotoxins in the food samples that may render severe threat to human health (Habiba et al., 2015). A study conducted by Şumălan et al. (2011) showed variable fungal growth in between $3.8 \times 10^4$ - $5.9 \times 10^4$ CFU/g in the food samples. Overall fungal growth was observed in only two samples which were much lower than the previous report (Table 1). Fungi are far and wide disseminated and seen wherever moisture is present with sufficient nutrients that can prolong their growth. Fungi are the foremost spoilage of foods and feedstuffs. The propagation of various fungi in

Table 1. Microbiological analysis of the various categories of food items

| Sample no | Sample                      | TVBC (CFU/g or CFU/mL) | TCC (CFU/g or CFU/mL) | TSC (CFU/g or CFU/mL) | TFC (CFU/g or CFU/mL) | Fecal coliform (CFU/g or CFU/mL) |
|-----------|-----------------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------------------|
| 1         | Fish (raw)                  | $4.6 \times 10^4$      | $1.6 \times 10^4$     | 0                     | 0                     | 0                                 |
| 2         | Fish (frozen)               | $3.1 \times 10^3$      | $8.3 \times 10^3$     | $2.2 \times 10^3$     | $2.9 \times 10^3$      | $2 \times 10^3$                   |
| 3         | Sea fish (raw)              | $1.94 \times 10^6$     | $8.1 \times 10^4$     | $2.6 \times 10^3$     | 0                     | 0                                 |
| 4         | Fish (shrimp frozen)        | $7.2 \times 10^4$      | $1.5 \times 10^4$     | $5.6 \times 10^3$     | 0                     | 0                                 |
| 5         | Meat (raw)                  | $1.76 \times 10^6$     | 0                     | $7.2 \times 10^5$     | 0                     | 0                                 |
| 6         | Meat (frozen)               | $8.2 \times 10^5$      | $1.2 \times 10^2$     | $4.5 \times 10^1$     | $4 \times 10^1$        | 0                                 |
| 7         | Sausage                     | $1.64 \times 10^7$     | $4.5 \times 10^6$     | $2.2 \times 10^5$     | 0                     | 0                                 |
| 8         | Yoghurt-1                   | $1.0 \times 10^4$      | 0                     | 0                     | 0                     | 0                                 |
| 9         | Yoghurt-2                   | $1.3 \times 10^4$      | 0                     | 0                     | 0                     | 0                                 |
| 10        | Yoghurt-3                   | $1.5 \times 10^4$      | 0                     | 0                     | 0                     | 0                                 |
| 11        | Ice cream-1                 | $1 \times 10^5$        | $1.1 \times 10^2$     | $3.8 \times 10^3$     | 0                     | 0                                 |
| 12        | Ice cream-2                 | $2 \times 10^4$        | $2.3 \times 10^2$     | $6.0 \times 10^2$     | 0                     | 0                                 |
| 13        | Pasteurized milk            | $4.4 \times 10^3$      | 0                     | 0                     | 0                     | 0                                 |
| 14        | Raw milk                    | $8.24 \times 10^4$     | $2.5 \times 10^5$     | $4.8 \times 10^4$     | 0                     | 0                                 |
| 15        | Commercial juice-1          | $3.2 \times 10^3$      | $2 \times 10^3$       | 0                     | 0                     | 0                                 |
| 16        | Commercial juice-2          | $3.7 \times 10^3$      | 0                     | 0                     | 0                     | 0                                 |
| 17        | Commercial juice-3          | $4.5 \times 10^3$      | 0                     | 0                     | 0                     | 0                                 |
| 18        | Commercial juice-4          | $2.0 \times 10^3$      | 0                     | 0                     | 0                     | 0                                 |

TVBC: Total viable bacterial count, TCC: Total coliform count, TSC: Total staphylococcal count, TFC: Total fungal coliform. Salmonella spp., Shigella spp., Vibrio spp., Pseudomonas spp. were completely absent in all the samples being studied.
agricultural products leads to lessening in yield and quality with noteworthy economic losses. The level of contamination will vary with geographic location, agricultural practices and the vulnerability of goods to the incursion of fungi during storage and processing periods (Adeyeye, 2016).

3.1.5 Antibiogram of the Isolates

Antibiotic resistance has augmented worldwide causing to failures in the treatment of human infectious diseases. Resistance against antibiotics by pathogenic bacteria is a major threat in the anti-infective therapy of both humans and animals. The Kirby-Bauer disk diffusion method was used in this experiment to resolve whether the isolated organisms were susceptible or resistant towards a pool of commonly prescribed antimicrobial agents. Antibiotic resistance pattern of E. coli, Klebsiella spp. and Staphylococcus spp. has been shown in Table 2. Both E. coli and Klebsiella spp. showed a high degree of sensitivity against Neomycin, Kanamycin, Gentamycin, Amikacin and Azithromycin. 40% of the Klebsiella spp. isolates were found to be resistant against cefixime. On the contrary, Staphylococcus spp. isolated from various samples exhibited 100% resistance against Cefixime. Similar type of resistance pattern in S. aureus isolates was also detected by Taj et al. (2010). Researchers have found that bacterial resistance to various antibiotics is encoded by over 100 different genetic mechanisms, a number of which are readily moveable to other bacteria through the process of conjugal elements, transposons, plasmids etc (Bhowmik et al., 2016).

4. Conclusion

It is obligatory that foods should be free of contaminations as much as possible. The existence of E. coli, Staphylococcus spp. and Klebsiella spp. indicates a potential health risk as these organisms are pathogenic and have been implicated in food borne diseases. Based on FDA guideline (2013), the level of contaminations was within unacceptable microbiological limits for four samples; this could be due to improper processing, poor handling practices and post-cross contamination which can produce a risk to the health of the consumers. Foodborne illness can be avoided by good hygiene practices such as the use of Hazard Analysis Critical Control Point (HACCP) application in the sequence of food production, processing and storage. Ensuring proper supervision and education to the food handlers/ food vendors on food safety practices and stringent control of various categories foods sold to the busy city residents should be properly observed by the pertinent authorities to ward off the food borne illness related epidemics within the Dhaka city, Bangladesh. Besides recurrent use of antibiotics should be prohibited as antibiotic defiant strains are incessantly mounting.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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| Name of Antibiotic disc | Disc Content | Zone of inhibition (mm) against test bacteria | E. coli (N = 10) | Klebsiella spp. (N = 10) | Staphylococcus spp. (N = 10) |
|------------------------|--------------|---------------------------------------------|-----------------|------------------------|----------------------------|
|                        |              | R%  | S%  | R%  | S%  | R%  | S%   |
| Neomycin               | 30 µg        | 0   | 100 | 10  | 90  | 3   | 70   |
| Cefixime               | 5 µg         | 0   | 100 | 40  | 60  | 100 | 0    |
| Kanamycin              | 30 µg        | 0   | 100 | 0   | 100 | 0   | 100  |
| Gentamycin             | 10 µg        | 10  | 90  | 0   | 100 | 0   | 100  |
| Amikacin               | 30 µg        | 15  | 85  | 0   | 100 | 10  | 90   |
| Ciprofloxacin          | 5 µg         | 10  | 90  | 5   | 95  | 0   | 100  |
| Azithromycin           | 25 µg        | 0   | 100 | 0   | 100 | 10  | 90   |

R% = Resistant percentage, S% = Sensitive percentage
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