

Microbiological analysis of ready to eat foods collected from different places of Dhaka city, Bangladesh

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The current study was attempted to observe the microbiological quality of ready to eat foods collected from different places of Dhaka city, Bangladesh. Ten food samples that include five fried items-fried chicken, fish fillet, shrimp fries, French fries, potato chop and five sweet items-yoghurt, pudding, ice cream, mango drink, lemon drink were analyzed during the period from September 2017 to November 2017. 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. The higher counts of TVBC, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus* spp. were recorded as 1.94×10^6 cfu/g (potato chop), 3.1×10^4 cfu/g (fried shrimp), 7.5×10^3 cfu/g (fried chicken) and 1.0×10^3 cfu/g (French fries) respectively. Based on the specifications by International Commission for Microbiological Specification for Foods (ICMSF), the level of contaminations was within acceptable microbiological limits except for potato chop.

Key words: Ready-to-eat foods, Microbiological analysis; Food safety

Ready-to-eat (RTE) foods have become more and more accepted in the last few years, specifically in metropolitan areas (1). Ready-to-eat (RTE) foods implies those foods that do not need further significant processing other than reheating or completion of a cooking process (2, 3). It has been found that RTE takeaway foods is dealing with a large volume of sales of the food service sector, representing more than a third of the food service volume outputs (4). Well known taste, cheap in price and convenience are some of the attracting factors that make RTE foods popular as food source. The RTE food products offer a source of readily available and wholesome meals for the consumer (5).

Food borne disease outbreaks linked with RTE foods have been linked with different types of foodborne pathogens (6, 7). The occurrence of foodborne illness is escalating worldwide (8, 9). The original microbiological load on RTE food ingredients is vital, however, factors such as handling, processing, storage and display may persuade the microbiological load of RTE foods at the point of sale (10). Ready to eat foods are often processed by hand and this direct contact may lead to an augmented incidence of contamination with potential food borne pathogens, such as *Staphylococcus* spp (11). The microbiology of RTE foods during preparation in factories, in domestic

kitchens, in canteens and on street corners by street vendors has formerly been examined (12).

Elevated counts of *Escherichia coli* and total coliform (TC) in foods usually implies lack of hygiene in handling and production operations, insufficient storage and post-process contamination (13). Therefore, *E. coli* and TC enumeration are used as a food-quality stricture. *Bacillus cereus* is commonly isolated from the natural environment (soil and growing plants) and foods, meat products, raw meat and meat product additives. *Salmonella* can repeatedly be isolated from raw foods of animal origin. Environmental contamination can also effect in *Salmonella* being present in a wide variety of foods, although generally at lesser numbers (14, 15).

Therefore, the capacity of development as well as the continued existence of bacteria must be monitored not only to notice the microbiological quality but also to evaluate the consumer welfare of such ready to eat food products (16). However, the rapid and accurate identification of foodborne pathogenic bacteria in food is important both for quality assurance and to detect pathogens within the food supply (17). Along these lines, current study focused on the presumptive detection of some common food borne pathogens among the ready to eat food samples.

MATERIALS AND METHODS

Sampling and sample collection The microbiological analysis of 10 ready to eat food items was categorized into two groups, of which five samples were fried types (fried chicken, fish fillet, shrimp fry, French fries, potato chop) and the rest five were sweet items (yogurt, pudding, ice cream, mango drink, lemon drink). These samples were collected from various vendor food shops of Dhaka city,

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Bangladesh within the period of January, 2017 to March, 2017. All the samples were transported to the laboratory instantly after collection in sterile plastic bags and kept them in ice box at 4 °C according to the method suggested by American Public Health Association (18).

Sample processing and enrichment of samples. In case of every sample, 10 g sample was weighted and then homogenized in 90 ml normal saline (NS) to make a 100 ml sample suspension for the microbiological examinations. For enrichment purposes, 1 ml of each sample was added to 9 ml of the selenite cysteine broth (SCB) ((Difco Laboratories, Detroit, Mich.) for both *Salmonella* and *Shigella* spp. Culture suspensions were incubated for 4 hours at 37 °C at 100 rpm (19).

Enumeration of total viable bacterial count (TVBC) and total coliform count (TCC). For the enumeration of total viable bacteria and coliforms (especially *E. coli* and *Klebsiella* spp.), an aliquot of 0.1 mL of each suspension was introduced onto the nutrient agar (NA) plates and MacConkey agar plates. After spreading 0.1 ml suspension from the dilution 10⁻³, the NA and MacConkey agar plates were incubated at 37 °C for 24 hours (19).

Detection of *Bacillus* spp., *Salmonella* spp., and *Shigella* spp. Starch agar plates were used to enumerate the contaminating *Bacillus* spp. within the examined samples while Salmonella-Shigella (SS) agar was used both for the isolation and enumeration of *Salmonella* and *Shigella* spp. After incubation at 37 °C for 24 hours, characteristic colonies were noticed and enumerated. Colonies with zone of starch hydrolysis followed by formation of starch iodine complex upon addition of iodine on the plate indicated the presence of *Bacillus* spp. while the black-centered colony while the black centered colony on Salmonella-Shigella agar was regarded as *Salmonella* spp. and the colorless colony on the same agar plates were noted as *Shigella* spp. (20).

Isolation of *Staphylococcus* spp., *Pseudomonas* spp. *Staphylococcus* & *Pseudomonas* spp. were isolated from the Mannitol Salt Agar (MSA) & Pseudomonas agar (PA) individually by spreading 0.1ml of the diluted samples on these media & then incubated at 37 °C for 24 hours (21).

Biochemical tests Identification of the isolates was done 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 (22).

RESULTS AND DISCUSSIONS

Foodborne diseases are the foremost global problem causing considerable morbidity and mortality each year (23). The most frequent known causes of foodborne diseases are pathogenic bacteria. In this study, the goal was to analyze the microbiological quality of RTE food produced by various vendor food shops within Dhaka metropolitan, Bangladesh. Therefore, the current study attempted to check the presence of microorganisms in various ready to eat 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.

Isolation and enumeration of microorganisms

Total Viable Bacterial Count (TVBC). In this study, the total viable bacterial counts ranged from 1×10³ cfu/g to 1.94×10⁶ cfu/g which were found in ice cream and potato chop respectively (Table 11). This finding was quite similar to the study conducted by Oranusi et al. (24). According to FDA guideline, 2013 (25), the acceptable limit is 5×10⁴–10⁵ cfu/g in case of total viable bacteria.

***E. coli*, *Klebsiella* spp., *Salmonella* spp. and *Shigella* spp. count.** Among all the ten samples, the highest *E. coli* count was observed as 3.1×10⁴ cfu/g in fried shrimp whereas the lowest count was 3.8×10² cfu/g in mango drink (Table 1). In this study, the detection of coliform bacteria, especially *E. coli* in four

Samples shows the possibility of the presence of fecally contaminated microorganism as also suggested by Adams and Moss (26). but in contrast with Tambekar et al. (27) *Salmonella* or *Shigella* species were not identified.

***Staphylococcus aureus* count.** *Staphylococcus aureus* count was observed within the range from 1.1×10² cfu/g – 7.5×10³ cfu/g which was found on French fries and fried chicken respectively (Table 1). The presence of *S. aureus* in RTE food is an indication of poor hygiene practices. *S. aureus* in RTE food is associated with cross contamination occurring during processing and storage or through the contamination of raw ingredients (28).

***Bacillus* spp. count.** *Bacillus* spp. count was noticed only in one sample. The count was recorded as 1.0×10³ cfu/g in case of French fries samples. The occurrence of *Bacillus* spp. in this study is similar as suggested by Rajkowski and Bennett (29) that these bacteria are coupled with the production of toxin; which causes food poisoning. These bacteria are available in dust, soil and raw food and can survives normal cooking as a heat resistant spore. These heat-resistant spores may have survived processing while vegetative cells were destroyed (27).

CONCLUSION

It is compulsory that foods must be free from contaminations as much as possible. The existence of *E. coli*, *S. aureus* and *Bacillus* spp. indicates a potential health risk as these organisms are pathogenic and have been implicated in food borne diseases (30, 31, 32). Based on the specifications by International Commission for Microbiological Specification for Foods (33), the level of contaminations was within acceptable microbiological limits except for potato chop; this could be attributed to improper processing, poor handling practices and post-cross contamination which can create risk to the health of the consumers. Foodborne illness can be barred by good hygiene practices such as the use of Hazard Analysis Critical Control Point (HACCP) application in the chain of food production, processing and storage. Ensuring proper guidance and education to the food handlers/ food vendors on food safety practices and strict control of ready-to-eat foods sold to the busy city inhabitants should be properly monitored by the relevant authorities to avert the epidemics of food borne illness within the Dhaka metropolitan area.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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TABLE 1. Isolation and enumeration of microorganisms found from ready to eat food samples

Sample	Total Viable Bacteria (cfu/g)	<i>S. aureus</i> (cfu/g)	<i>Escherichia coli</i> (cfu/g)	<i>Klebsiella</i> spp. (cfu/g)	<i>Salmonella</i> spp. (cfu/g)	<i>Shigella</i> spp. (cfu/g)	<i>Bacillus</i> spp. (cfu/g)
Fried Chicken	1.4×10 ⁴	7.5×10 ³	0	0	0	0	0
Fish fillet	2.5×10 ⁵	1.30×10 ²	0	0	0	0	0
Shrimp fry	5.4×10 ⁴	2.2×10 ³	3.1×10 ⁴	0	0	0	0
French Fries	1.0×10 ⁵	1.1×10 ²	3.8×10 ³	0	0	0	1.0×10 ³
Potato Chop	1.9×10 ⁶	2.6×10 ³	1.1×10 ⁴	0	0	0	0
Yoghurt	5.0×10 ⁵	0	0	0	0	0	0
Pudding	3.3×10 ³	0	0	0	0	0	0
Ice cream	1.0×10 ³	2.0×10 ²	0	0	0	0	0
Mango drink	4.8×10 ⁵	4.0×10 ³	3.8×10 ²	0	0	0	0
Lemon drink	4.4×10 ⁵	6.0×10 ³	0	0	0	0	0

TABLE: 2. Biochemical identification of the bacterial isolates from different ready to eat food samples

Assumed Organism	TSI			H ₂ S creation	Indole test	MR test	VP test	Citrate Test	Motility
	slant	Butt	Gas						
<i>Staphylococcus aureus</i>	Y	Y	-	-	-	+	-	-	-
<i>Escherichia coli</i>	Y	Y	+	-	+	+	-	+	+
<i>Bacillus</i> spp.	-	-	-	+	-	-	+	+	+

TSI = Triple Sugar Iron; Y = Yellow (Acid); R = Red (Alkaline); MR = Methyl red; VP = Voges-Proskauer

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