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Evaluation of microbial contamination level and the drug susceptible pattern of the isolates cultivated from famous dessert food

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Abstract

Present study endeavored to evaluate the microbial contamination level along with their drug resistant pattern in some popular desert food items collected from different food shops in Dhaka city, Bangladesh. The microbial evaluation was conducted through conventional cultural methods and drug susceptibility test was executed through disc diffusion method. All the samples were found to be contaminated with heterotrophic bacteria as well as fungi within the range of 10^3 to 10^5 CFU/g. In case of specific microflora, the growth of *Staphylococcus* spp. was very high in sweet, faluda, milk cake and ice cream as estimated up to 10^5 CFU/g, halua and sweet yogurt showed 10^4 CFU/g while rest of the samples revealed 10^3 CFU/g. *E. coli* was found only in faluda and ice cream up to 10^3 CFU/g whereas *Klebsiella* spp. was estimated in all the samples within the range of 10^2 CFU/g to 10^5 CFU/g. *Salmonella* spp., *Pseudomonas* spp. and *Bacillus* spp. were totally absent in all the samples. Most of the isolates were found to be resistant against most of the antibiotics. Meanwhile, streptomycin (10 µg), gentamicin (10 µg), azithromycin (15 µg), and nalidixic acid (5 µg) were effective drug against both *E. coli* and *Staphylococcus* spp.

1. Introduction

Dessert is a sweet confectionary, which is not only very famous in Asian people but also it has huge demand in outside of the Asia as the last test of a meal. This definition includes a range of courses ranging from fruits or dried nuts to multi-ingredient cakes and pies. Many cultures have different variation on dessert. In modern times, the variations of desserts have usually been passed down or come from geographical regions. Especially, peoples are very used to have dessert in traditional cuisines or cultural programmes. Mainly, dessert are usually formulated by milk, sugar, modified starch, hydrocolloids like carrageenan, flavorings and colorants (De Wijk *et al*., 2003; González-Tomás and Costell, 2006). In case of puddings, sodas, cakes, ice cream, fruits, and pastries, the food makers are preferably using the whipped toppings. Therefore, it is important to maintain the quality of the products to eliminate the rate of food spoilage as well as food borne diseases such as diarrhea, dysentery, and abdominal cramping (Danielsson-Tham *et al*., 2004; MacDonald *et al*., 2005). Several reports have been conducted earlier on the transmission of diseases causing microbes especially *E. coli*, *Staphylococcus* spp., *Campylobacter*, *Klebsiella* spp. *Enterobacter*, etc. from the dairy-based foods to the human body especially in countries where hygienic standard is not up to the satisfactory level (Meyer-Broseta *et al*., 2003; Danielsson-Tham *et al*., 2004; MacDonald *et al*., 2005; Makino *et al*., 2005; Okwumabua *et al*., 2005; Oliver *et al*., 2005). The dried milk products must be stored in optimal conditions. If there is proper packaging, there will be no change in color, even during two years of storage at 35°C. Nevertheless, in commercial situations, most dry milk products are vulnerable to reactions, which will lead to small changes within the physical properties of the merchandise, its palatability and nutritive value. These

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changes, however, do not significantly influence the nutritional benefits of milk powders. Vitamin and protein quality losses during storage of milk powders, when stored in good conditions, are negligible (U.S. Dairy Export Council, 2001). Similarly, the quality of food depends on the general number of viable organisms as revealed by the general bacterial count. However, the load of microbes in food products is influenced by the type of things just like the final environment from which the raw materials were obtained, the environment during which it had been processed, the sanitary conditions under which the food was handled and processed, and so the adequacy of processing procedures targeted at reducing contaminants during the packaging, handling and storage of the merchandise (Osamwonyi, 2005). A significant group of contaminants within the dairy industry is caused by thermophilic bacilli. However, these bacilli are generally not pathogenic, their presence in dairy products is an indicator of poor hygiene and high numbers are unacceptable to customers. Additionally, their growth may end in milk product defects caused by the assembly of acids or enzymes, potentially leading to off-flavours. Dairy thermophiles (*Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni*) are usually selected for the conditions during dairy manufacture. For being a spore forming bacteria they are able to grow in dairy manufacturing plants where even temperatures reach 40–65°C.

Furthermore, the control of food contamination by microorganisms or the practice of food protection largely depends on the knowledge on types and modes of food hazards, the pathogenic trait, the virulence factors of the food contaminating bacteria and fungi, knowledge on toxins associated with food deterioration and finally the urge of practical implementation of food protection means both by the governmental or non-governmental organizations (NGOs). The main goal of the present study was to investigate the microbiological quality analysis of the dessert samples.

2. Materials and methods

2.1 Study area, sampling, sample processing and microbiological analysis

A total of eighteen different dessert (sweet, faluda, ice cream, pudding, halua, sweet yogurt, mango lassi, tusha haiwa, lemon curd, egg haloa, fresh cheese dessert, shemai, bread pudding, milk cake, jellow fruit custard, faluda, cake and rasmalai) were randomly collected from commercial shop in Dhaka City, Bangladesh following standard protocol (APHA 1998). All the samples were quickly transported to the laboratory. Prior to microbiological assay, 10 g of each sample was homogenized with 90 mL of distilled water 9:1 ratio and serially diluted in normal saline up to 10^{-3}.

From the dilution 10^{-2} of each of the samples, 0.1 mL was introduced on to the nutrient agar and Sabouraud dextrose agar for the isolation of total viable bacteria and fungi, respectively. Subsequently, MacConkey agar, Membrane Fecal Coliform agar (M-FC), Mannitol Salt agar, *Pseudomonas* agar, *Starch agar* and XLD agar were used as selective media for the quantification of coliforms, fecal coliforms, *Staphylococcus* spp., *Pseudomonas* spp. *Bacillus* spp. and *Salmonella* spp., respectively (Cappuccino and Sherman, 1996; Acharjee, Fatema, Jahan *et al*., 2013; Hassan *et al.,* 2013 Acharjee *et al*., 2014). All the inoculated plates were incubated at 37°C for 24 hours except SDA plates, which were incubated at 25°C for 48 hrs.

2.2 Taxonomic identification of the isolates

The biochemical properties of identified isolates were confirmed through standard biochemical methods such as triple sugar iron test, motility test, indole production, methyl red reaction, Voges-Proskauer test, citrate utilization, catalase test and oxidase test (Cappuccino and Sherman, 1996; Islam *et al*., 2020).

2.3 Antibiotic susceptibility test of the identified bacteria

The pathogenic isolates were examined for antibiotic susceptibility traits (either drug resistant or sensitive) by using disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against commonly used antibiotics following the standard protocol (Bauer *et al*., 1966; Ferraro, 2001; Nur *et al*., 2020). A thin layer of bacterial growth such as *Escherichia coli*, *Pseudomonas* spp., *Vibrio* spp., *Staphylococcus* spp. and *Salmonella* spp. (turbidity compared with the McFarland standard OD_{600}-0.5) were subjected to lawn on to surface of Muller Hinton agar. Antibiotics used in the study included polymyxin B (300 unit), kanamycin (30 µg), methicillin (30 µg), streptomycin (10 µg), vancomycin (30 µg), gentamycin (10 µg), nalidixic acid (30 µg), azithromycin (15 µg), penicillin G (10 µg), erythromycin (15 µg), amoxicillin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg) and cefixime (5 µg). All plates were incubated at 37°C for 12-18 hrs and examined for formation of the zone of inhibitions (mm).

3. Results and discussion

3.1 Microbial load in dessert samples

All the samples were found to be contaminated with

total viable bacteria (TVB) as well as fungi. In case of TVB the growth was estimated within the range of 10^2 to 10^5 CFU/g while the fungal contamination was noticed in between 10^2 to 10^4 CFU/g Table 1). The growth of *Staphylococcus* spp. was very high up to 10^5 CFU/g in sweet, faluda, milk cake and ice cream, in halua and sweet yogurt 10^4 CFU/g was found while rest of the samples exhibited up to 10^3 CFU/g. *E. coli* was found only in two samples faluda and ice-cream up to 10^2 CFU/g while *Klebsiella* spp. was estimated in all the samples within the range of 10^2 CFU/g to 10^5 CFU/g. *Salmonella* spp., *Pseudomonas* spp. and *Bacillus* spp. were totally absent in all the samples. As described in many previous studies that the undesired microflora may transmitted in food samples during the processing of raw material, mixing of ingredients and packaging of the end products (Institute of Food Technologists, 2000; FAO and WHO, 2003; European Commission, 2004; Center for Food Safety and Applied Nutrition, (2008); Jane *et al.,* 2018; FSANZ, 2001). According to the International Commission on Microbiological Specifications for Foods (ICMSF) 2011 the presence of specific pathogen in food should not be exceeded the marginal limit 10^3 CFU/g. In this study, most dessert harbored excessive amount of Staphylococcal growth which exited the marginal limit provided by International Commission on Microbiological Specifications for Foods (ICMSF) 2011.

3.2 Biochemical properties of the isolates

All the isolates showed their physiological and metabolic activity through several biochemical tests (Table 2).

3.3 Proliferation of drug-resistant bacteria in dessert food samples.

To evaluate the efficacy of commonly available antibiotics as well as the clinical significance of the bacterial isolates, present study introduced antibiotic susceptibility test. Identified bacterial isolates were experimented to determine the antibiotic susceptibility against the commonly antibiotics. Most of the isolates were found to be resistant against most of the antibiotics, only streptomycin (10 µg), gentamycin (10 µg), azithromycin (15 µg), and nalidixic acid (5 µg) were effective drug against both *E. coli* and *Staphylococcus* spp. (Table 3).

As described in previous research that such drug resistance properties of bacteria may increases the host immunity as well as the beneficial role of host normal flora which can directly hinder the proper medication (Bennett, 2008; Acharjee, Jahan, Rahman *et al*., 2013). Several reasons are responsible to increases the bacterial resistance such as genetic alteration through mutation,

Table 1. Microbiological profiling of sampled dessert.

Sampled Dessert	TVB	Fungi	*E. coli*	*Klebsiella* spp.	*Bacillus* spp.	*Pseudomonas* spp.	*Salmonella* spp.	*Staphylococcus* spp.
Sweet	2.0×10^5	2.0×10^3	0	7.0×10^2	0	0	0	2.8×10^5
Faluda	4.0×10^5	2.7×10^3	3.7×10^2	5.0×10^5	0	0	0	4.3×10^5
Ice-cream	7.5×10^5	4.7×10^4	2.1×10^2	4.5×10^3	0	0	0	7.0×10^5
Pudding	2.5×10^4	0	0	8.8×10^2	0	0	0	2.9×10^3
Halua	2.0×10^4	2.1×10^3	0	6.0×10^5	0	0	0	2.8×10^4
Sweet yogurt	2.0×10^3	1.5×10^2	0	4.8×10^2	0	0	0	2.5×10^4
Mango Lassi	3.0×10^3	2.0×10^3	0	4.4×10^3	0	0	0	2.3×10^2
Tusha Haiwa	4.0×10^5	2.0×10^3	0	2.5×10^3	0	0	0	2.0×10^3
Lemon Curd	2.0×10^3	1.5×10^2	0	3×10^2	0	0	0	2.3×10^3
Egg Haloa	2.5×10^3	2.5×10^3	0	9.3×10^2	0	0	0	4.2×10^3
Fresh cheese dessert	2.0×10^3	2.0×10^3	0	4.5×10^3	0	0	0	2.7×10^2
Shemai	4.0×10^5	1.5×10^2	0	3.0×10^5	0	0	0	2.0×10^3
Bread pudding	2.5×10^4	0	0	2.5×10^3	0	0	0	2.5×10^3
Milk cake	2.0×10^5	1.5×10^2	0	4.8×10^2	0	0	0	2.2×10^5
Jellow fruit custard	2.0×10^3	1.5×10^2	0	6.0×10^5	0	0	0	2.5×10^2
Falooda	3.0×10^2	2.7×10^3	0	2.0×10^2	0	0	0	4.2×10^3
Cake	2.0×10^3	1.5×10^2	0	3.0×10^5	0	0	0	3.0×10^2
Ras malai	2.0×10^3	2.7×10^3	0	4.5×10^3	0	0	0	2.5×10^3

Table 2. Biochemical identification of the isolates

Assumed Pathogenic microorganisms	TSI				Motility	Indole Production	MR	VP	Citrate utilization	Catalase	Oxidase
	Slant	Butt	Gas	H_2S							
E.coli	Y	Y	+	-	+	+	+	-	-	+	-
Klebsiella spp.	Y	Y	+	-	+	-	-	-	+	+	-
Staphylococcus spp.	Y	Y	-	-	+	-	+	-	+	+	-

TSI: Triple Sugar Iron Test, Y: Yellow (Acid), R: Red (Alkaline), MR: Methyl red, VP: Voges-Proskauer

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Table 3. Antibacterial susceptibility test of the isolates

Antibiotic	*E. coli* (n = 2)		*Klebsiella* spp. (n = 18)		*Staphylococcus* spp. (n = 18)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Polymyxin B (300 unit)	100	0	100	0	100	0
Kanamycin (30 µg)	100	0	100	0	100	0
Methicillin (30 µg)	100	0	100	0	100	0
Streptomycin (10 µg)	0	100	0	100	0	100
Vancomycin (30 µg)	100	0	100	0	100	0
Gentamycin (10 µg)	0	100	0	100	0	100
Nalidixic acid (30 µg)	0	100	0	100	0	100
Azithromycin (15 µg	0	100	0	100	0	100
Penicillin G (10 µg)	100	0	100	0	100	0
Erythromycin (15 µg)	100	0	100	0	100	0
Amoxicillin (30 µg)	100	0	100	0	100	0
Ceftriaxone (30 µg)	100	0	100	0	100	0
Ciprofloxacin (5 µg)	100	0	100	0	100	0
Ampicillin (10 µg)	100	0	100	0	100	0
Tetracycline (30 µg)	100	0	100	0	100	0
Chloramphenicol (30 µg)	100	0	100	0	100	0
Cefixime (5 µg)	100	0	100	0	100	0

R: resistant, S: sensitive

abuse of antibiotics, short-term drugs ingestion and easy access to get drugs from retailer without prescription (Canteón, 2009; Islam *et al.,* 2020).

4. Conclusion

Cross-contamination of foods is one of the major concerns in the food industry, and if microorganisms are not completely removed from food-contact surfaces they may go on to form biofilms and increase the bio-transfer potential. In conclusion, this study demonstrates the presence of some pathogens including *Staphylococcus* spp., *Klebsiella* spp. and *E. coli* in dessert. Therefore, these foods are serious risk to the public health. Likewise, the presence of these organisms indicated that there were poor hygienic conditions during the manufacturing, storage and sales process of these traditional foods. Manufacturing procedures within the scope of the HACCP, appropriate hygienic measures to avoid pre-processing and post processing cross contamination and the use of pasteurized milk are critical to control such types of drug resistant pathogens in food samples. It is now very urge to find out the route of cross contamination in food (from raw material processing to final packaging) by which we can reduce such undesired growth of microorganisms in the food items.

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