

Isolation of pathogenic microorganisms and determination of their antibiotic resistance patterns collected from different bakery products of Dhaka city

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Abstract

Bakery products play an important part in the diet of daily life and provide vital nutrients to human. But pathogenic microorganisms may get entry to bakery items through improper handling and storage which may lead some disease to consumers. For this reason, the objective of this study was to detect pathogenic bacteria in different bakery products collected from different places of Dhaka city. In this study, commonly consumed bakery items such as sweets, biscuit, cake and bread samples were collected from different locations for the detection of pathogenic microorganism. Here, all the samples were found to be contaminated huge microbial count up to 10^6 CFU/g. *Klebsiella* spp. and *Pseudomonas* spp. were most predominate among all. On the other hand, *Escherichia coli* was totally absent in all the samples. This result indicated that bakery products prepared under unhygienic environments and which may serve as a reservoir of various pathogenic bacteria and most of them were showed resistance against common antibiotics. To ensure the health safety of consumer's government should take necessary actions to educated food handlers.

1. Introduction

Bakery products are most consumed food worldwide and a good source of different essential nutrients such as carbohydrates, proteins, lipids, vitamins and minerals (Potter and Hotchkiss 2006; Saranraj and Geetha, 2012). As they contain low water activity and cooked under high temperature freshly baked products are free from microbes and do not contain pathogenic microorganisms therefore from the microbiological point of view bakery products are generally considered as safe food (ICMSF 2002; Saddozai and Khalil, 2009; Saranraj and Geetha 2012; Khanom *et al.*, 2016). Cooking method, lower pH, the addition of preservative and low moisture in bakery items can prevent products from bacterial spoilage and also extended their self-life (Berghofer *et al.*, 2003).

But improper storage conditions, unhygienic handling may support the proliferation of various microorganisms in cereal grains and bakery products (Deibel and Swanson, 2001). Some common bacteria like *Bacillus* sp. and mold include *Rhizopus* sp., *Mucor* sp., *Penicillium* sp., *Eurotium* sp., *Aspergillus* sp. and *Monilia* sp. are responsible for spoilage which depends on seasons, type of products and methods of processing (Seiler, 2000; Saranraj and Geetha 2012). Several studies conducted in various countries have reported that food

poisoning resulting from contaminated flour (Saddozai and Khalil, 2009; Saranraj and Geetha 2012; Khanom *et al.*, 2016). But others research on wheat and flour were reported that they contain pathogenic microorganisms like *Salmonella* spp., *Escherichia coli*, *Bacillus cereus* and other spoilage micro-organisms in low levels (Richter *et al.*, 1993; Victor *et al.*, 2013). So, it would be different physical, chemical and microbial factor-like slicing equipment, post-baking contamination from the handler, bread coolers, conveyor belts, racks and high moisture can also influence the spoilage of bakery products (Saddozai and Khalil, 2009; Saranraj and Geetha 2012). Bakery products come in contact with moulds, yeasts and bacteria such as the rope-causing heat - resistant endospore-forming *Bacillus subtilis* during production and inappropriate storage (Earle and Putt, 1984). Mold spores are killed in the baking process (Knight and Menlove, 1961), leaving often contamination to be the source of spoilage problems. *Penicillium* is the most common fungal contaminants which also necessary for sourdough bread (Legan and Voysey, 1991). Some bacterial spore-like *Bacillus* spores are very dangerous because of its ability to survive in high temperature also in the baking period which may be germinated after baking or during storage (Legan, 1993). Several studies on food reported that contaminated and

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unhygienic products have been associated with food-borne illness, for this reason, analyses of bakery products are essential as these may affect the overall health of the persons and consumers (Saranraj and Geetha, 2012).

The objective of this study was to investigate the microbiological quality of the bakery samples collected from the market of Dhaka city and determination of antibiotic resistance patterns of isolated pathogens.

2. Materials and methods

2.1 Study area, sampling and sample processing

Different bakery products were collected from markets early in the morning after production. All samples were instantly transported to the Microbiological Laboratory at Stamford University Bangladesh, Dhaka. Each sample (10.0 g) was homogenized with 90.0 mL of sterile normal saline to prepare a stock solution. Stocks were serially diluted up to 10^{-5} by adding 1 mL of samples on a series of 9 mL of normal saline.

2.2 Microbiological analysis of each sample

A volume of 0.1 mL from each sample suspension was spread onto nutrient agar (NA) and incubated at 37°C for 24 hrs for enumerating total viable bacteria (TVB). Sabouraud dextrose agar (SDA) (Oxoid Ltd., Basingstoke, Hampshire, England) was inoculated followed by incubation at 25°C for 48 hrs for the isolation of fungi (Noor et al. 2013; Rahman et al., 2016).

2.3 Isolation pathogenic microorganisms

For the isolation of coliform bacteria (*Escherichia coli*, *Klebsiella* spp.), 0.1 mL of each sample suspension was spread over MacConkey (Oxoid Ltd., Basingstoke, Hampshire, England) agar and incubated at 37°C for 24 hours.

0.1 mL of each sample suspension was spread on mannitol salt agar (MSA) (Oxoid Ltd., Basingstoke, Hampshire, England) for the estimation of *Staphylococcus aureus* and the plates were incubated at 37°C for 24 hrs. For the estimation of starch hydrolyzing bacteria (*Bacillus* spp.), 0.1 mL of each sample suspension was spread onto starch agar (SA) (Oxoid Ltd., Basingstoke, Hampshire, England) and incubation at 37°C for 24 hrs.

For the enumeration of *Pseudomonas* spp., 0.1 mL of each sample suspension was spread onto *Pseudomonas* agar (Oxoid Ltd., Basingstoke, Hampshire, England) and plates were incubated at 37°C for 24 hrs. All the microorganisms were further confirmed by biochemical

methods (Cappuccino and Sherman, 1996; Rahman et al., 2016; Noor et al., 2013; Marjan et al., 2014; Rahman et al., 2016; Rahman et al., 2016).

2.4 Enrichment of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp.

The *in vitro* cultivation of the species of *Salmonella*, *Shigella* and *Vibrio* often appears difficult or with faulty results (false-negative) due to their viable but non-culturable (VBNC) attributes (Colwell, 2000; Oliver, 2005; Das et al., 2013). Consequently, enrichment was used prior to isolating these bacteria (Rahman and Noor, 2012; Ahmed et al., 2014). For the isolation of *Salmonella* spp. and *Shigella* spp., 1 mL of homogenized sample suspension was transferred to selenite cystine broth (SCB) and for *Vibrio* spp. 1 mL of sample was transferred to alkaline peptone water (APW) then incubated at 37°C for 4-6 hrs. After incubation samples were serially diluted up to 10^{-4} and from 10^{-4} dilution 0.1 mL was spread onto *Salmonella* Shigella (SS) agar (Hi media, India) and TCBS (Hi media, India) respectively for *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. (Noor et al., 2013; Rahman et al., 2016).

2.5 Antibiotic susceptibility test

Antibiotic susceptibility traits of the pathogenic isolates were examined by disc diffusion method where bacterial suspension (10^5 cells or 0.5 OD) were prepared from isolated pathogens. Then bacterial lawn was done on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England) plates and commercially available laboratory-grade antibiotic discs (Ampicillin (10 µg), Ciprofloxacin (5 µg), Streptomycin (10 µg), Ceftriaxone (30 µg), Imipenem (30 µg), Penicillin (10 µg), Gentamycin (10 µg), Azithromycin (15 µg), Tetracycline (30 µg), Cefixime (5 µg), Erythromycin (15 µg) and Trimethoprim-Sulfamethoxazole (25 µg) were aseptically placed on the surface of agar plates at spatial distance of 5 mm (Marjan et al., 2014).

3. Results and discussion

Different studies have observed that bakery products are an important portion of energy intake (Agte et al., 2002; Bartrina et al., 2004; Vanelli et al., 2005). But post preparation contamination is a serious problem for public health. Food safety authority's make some food standard the maximum permissible limits in baked products (cake, bread and biscuits) for total plate count (TPC) is $<10^5$ CFU/g, yeast and mold is $<10^4$ CFU/g, coliform bacteria <200 CFU/g, *E. coli* absent (Gilbert et al., 2000; Hocking, 2003; NSW Food Authority, 2009).

The present study revealed that all the samples were

found to be contaminated with heterotrophic bacteria within the range of 10^4 to 10^7 CFU/g (Table 1). Six out of twenty samples exceeded the microbial limit. The proliferation of bacteria may lead to disease if bakery items consumed by immune-compromised patient or children which is a serious problem for the buyer. In the case of total fungi, all the bakery items were found to harbor microorganisms within the range of 10^2 to 10^3 CFU/g which is under the microbial limit.

In the case of specific microflora the growth of *Klebsiella* spp., *Pseudomonas* spp. are most predominate those are confirmed by biochemical identification (table 2). *Klebsiella* spp. are found on all the samples within a range of 10^2 - 10^5 CFU/g which is very alarming which also sheds light on the unhygienic processing conditioning. *Pseudomonas* spp. is ubiquitous organisms which can be found everywhere, but the presence in food was not acceptable. Here, out of ten samples, only six contain *Pseudomonas* spp. *E. coli* was totally absent in all the samples. Among the four samples, most of the bread and cake were contaminated with *Staphylococcus* spp. Therefore, it can be inferred that these baked products were not handled in an appropriate manner by the workers. Consuming *Staphylococcus* spp. also can cause diseases such as nausea and vomiting, they should use gloves and do not use outside clothing in possessing areas. Surprisingly, *Salmonella* spp. also found to be harboring in two samples, which may come from poultry products like eggs or milk (Table 1). In the bakery, they

use egg in cake, bread and biscuits which major source of *Salmonella* spp. so egg should wash before formulation. *Vibrio* spp. was also present in three bakery items and water used in bakery may the possible source of these types of result.

Unacceptable levels of bacteria might result from this study may be due to poor hygienic practices, from dirty equipment and polluted environments of processing and storage area. Compare to other sweets items harbor less microbial count may be due to its high sugar content. Prevalence of drug-resistance gene is growing day by day within the human body through the ingestion of drug-resistant bacteria by water, food and pharmaceuticals (Ahmed *et al.*, 2013; Hassan *et al.*, 2013). A different study on food water and pharmaceuticals products revealed that they contain drug-resistant bacteria (Noor *et al.*, 2013; Ahmed *et al.*, 2013; Marjan *et al.*, 2014). In this study similarly, all pathogenic isolates were resistant against multiple antibiotics tested (Table 3). Among the five pathogens *Staphylococcus* spp. and *Pseudomonas* spp. exhibited more resistance against tested antibiotics which indicates them as the MDR strain. However, other pathogenic strain also contains resistant genes (Table 3). Abuse use of antibiotics may be a possible reason behind the development of drug-resistant strain which may generate serious health threat for consumers (Marjan *et al.*, 2014).

Table 1. Microbiological analysis of some bakery items

Sample	TVB	Total fungus	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Staphylococcus</i> spp.	<i>Vibrio</i> spp.	<i>Salmonella</i> spp.	<i>Pseudomonas</i> spp.
Baily road	Sweet	9.2×10^4	2.4×10^2	0	1.0×10^2	0	0	0
	Biscuit	6.5×10^4	2.6×10^2	0	5.0×10^2	1.7×10^2	0	0
	Cake	5.6×10^5	2.2×10^2	0	1.3×10^1	1.2×10^2	0	0
	Bread	2.1×10^6	2.6×10^3	0	1.2×10^1	1.5×10^2	0	0
Shantinagar	Sweet	3.4×10^4	4.6×10^2	0	0	0	0	0
	Biscuit	2.4×10^5	5.4×10^3	0	1.2×10^2	0	0	0
	Cake	7.5×10^5	1.5×10^2	0	1.1×10^2	1.7×10^2	0	8.8×10^2
	Bread	6.5×10^6	2.8×10^3	0	1.3×10^2	2.1×10^2	0	3.0×10^2
Mouchak	Sweet	1.9×10^4	5.6×10^3	0	0	0	2.0×10^2	0
	Biscuit	2.4×10^5	4.8×10^3	0	0	9.5×10^3	3.8×10^3	0
	Cake	5.3×10^5	3.2×10^2	0	3.6×10^3	2.5×10^2	0	0
	Bread	4.1×10^6	5.2×10^3	0	2.3×10^2	1.8×10^2	0	0
Khilgoan	Sweet	1.1×10^3	4.6×10^2	0	0	0	0	0
	Biscuit	1.9×10^4	1.2×10^3	0	0	0	0	0
	Cake	8.6×10^5	3.4×10^2	0	3.3×10^2	2.1×10^2	0	8.0×10^2
	Bread	3.9×10^6	4.4×10^3	0	5.6×10^1	2.2×10^3	0	4.1×10^2
Siddeshwari	Sweet	2.9×10^4	1.2×10^2	0	2.0×10^1	0	0	0
	Biscuit	2.8×10^5	1.7×10^2	0	0	0	0	0
	Cake	1.9×10^7	2.4×10^3	0	2.0×10^2	3.3×10^2	2.2×10^3	5.2×10^3
	Bread	1.8×10^7	2.1×10^3	0	1.3×10^2	4.0×10^2	7.5×10^2	3.1×10^3

Table 2. Biochemical reaction chart of different isolates to identify different bacteria

Assumed Organism	TSI			H ₂ S	Indole	MR	VP test	Citrate test	Motility	Oxidase test
	slant	butt	gas	creation	test	test				
<i>Klebsiella</i> spp.	Y	Y	+	-	-	-	+	+	-	
<i>Vibrio</i> spp.	Y	Y	-	-	+	+	-	+	+	+
<i>Salmonella</i> spp.	R	Y	-	+	-	+	-	-	+	
<i>Pseudomonas</i>	R	R	-	-	-	-	-	+	+	+

Y: Yellow (Acid), R: Red (Alkaline), MR: Methyl red, VP: Voges-Proskauer

Table 3. Antibiogram of different isolates from bakery products.

Antibiotics	<i>Klebsiella</i> spp.		<i>Staphylococcus</i> spp.		<i>Vibrio</i> spp.		<i>Salmonella</i> spp.		<i>Pseudomonas</i> spp.	
	n = 14		n = 11		n = 3		n = 2		n = 6	
	R	S	R	S	R	S	R	S	R	S
AMP (10µg)	7.50%	92.80%	90.90%	9.40%	66.60%	33.40%	0%	100%	83.30%	16.70%
CIP (5µg)	0%	100%	100%	0%	0%	100%	0%	100%	83.30%	16.70%
STE(10µg)	0%	100%	54.50%	45.50%	66.60%	33.40%	0%	100%	66.60%	33.40%
CEF (30µg)	28.60%	71.40%	81.80%	18.20%	33.30%	66.70%	0%	100%	50%	50%
IPM (30µg)	35.80%	64.20%	100%	0%	66.60%	33.40%	50%	50%	66.60%	33.40%
PEN (10µg)	57.20%	42.80%	100%	0%	100%	0%	100%	0%	83.30%	16.70%
GEN(10µg)	0%	100%	0%	100%	0%	100%	0%	100%	16.70%	83.30%
AZI (15µg)	28.60%	71.40%	63.60%	36.40%	0%	100%	50%	50%	33.40%	66.60%
TER (30µg)	0%	100%	36.40%	63.60%	33.40%	66.60%	50%	50%	50%	50%
CFX (5µg)	57.20%	42.80%	72.70%	27.30%	33.40%	66.60%	100%	0%	50%	50%
ERY (15µg)	57.20%	42.80%	90.90%	9.40%	33.40%	66.60%	50%	50%	66.60%	33.40%
SXT (25µg)	0%	100%	9.40%	90.90%	33.40%	66.60%	50%	50%	16.70%	83.30%

AMP = Ampicillin; CIP = Ciprofloxacin; STE = Streptomycin ; CEF = Ceftriaxone ; IPM = Imipenem ; PEN = Penicillin ; GEN = Gentamycin ; AZI = Azithromycin ; TER = Tetracycline ; CFX = Cefixime ; ERY = Erythromycin ; SXT = Trimethoprim-Sulfamethoxazole.

4. Conclusion

The present study revealed the microbiological and hygienic status of filled bakery products. Overall, the microbiological status of filled bakery products was slightly good while other samples were found to be contaminated with different microbes including disease causing bacteria with higher counts than accepted limits. The presence of those microbes in bakery products indicated an alarming situation for the final consumer. So, maintenance of hygienic practice during preparation of bakery products and use of standard procedure to reduce the cross contamination from raw materials is also necessary to ensure public health safety. However, the food quality in Bangladesh needs to be monitored regularly by BSTI and by the Ministry of Health in Bangladesh.

Conflict of Interest

The authors declare no conflict of interest.

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