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MICROBIOLOGICAL QUALITY OF CHEESE FOUND IN BANGLADESH

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Abstract

Cheese is a solid food, made from curd obtained from milk by coagulating the casein with the help of rennet or similar enzymes in the presence of lactic acid produced by the added or adventitious microorganisms. Cheeses, especially fresh and soft or semi-soft styles, are susceptible to contamination with pathogens such as *Shigella, Listeria monocytogenes*, *Klebsiella*spp. and with coliforms specially *Escherichia coli, Bacillus* spp., *Staphylococcus* spp. etc. The purpose of the current research was to investigate the µg)pathogenic organisms present in soft cheese available in supershops of Bangladesh. Two cheese samples were collected from Supershop-B &Supershop-A area of Dhaka city. Samples from Supershop-B market had high bacterial load (3.45×10^6cfu/ml) than those (2.22×10^5 cfu/ml) from Supershop-A market. The presence of total coliform (2.5×10^7cfu/ml) indicated that other harmful and pathogenic microorganisms such as *Klebseilla,*, *Staphylococcus aureus*, etc. might be present in the samples .The antibiotic susceptibility test was done by Kirby-Bauer method.*E.coli*strains *isolated* from cheese samples were almost sensitive against Gentamycin (10 µg), Ceftriaxone (30 µg), and Ciprofloxacin (5 µg), and were resistant against Ampicillin (10 µg) and Chloramphenicol (30 µg). *Listeria*, isolated from cheese samples were found resistant against Ampicillin, Gentamycin, Chloramphenicol and Ceftriaxone and the *Bacillus* sp. were found almost sensitive against Chloramphenicol and Ciprefloxacin, and were resistant against Ampicillin and Ceftriaxone.

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INTRODUCTION

Cheese can be roughly defined as a coagulated milk product. It is made by introducing bacteria or enzymes into the milk to separate the actual curds (milk solids) from the whey (liquids). the cheesemaker is to convert milk into cheese. The milk may be from a cow, goat, sheep or buffalo, although worldwide cow's milk is most commonly used. The cheesemaker applies craft and skill to the practise of cheesemaking, intending to produce a product with specific characteristics and organoleptic requirements that are consistent every time it is made. Some cheeses may be deliberately left to ferment from naturally airborne spores and bacteria; this generally leads to a less consistent product but one that is highly valuable in a niche market for exactly that reason, no two are ever quite the same (Robinson &Wilbey, 1998). Specific Temperature required promoting the growth of the bacteria that feed on lactose and thus ferment the lactose into lactic acid. These bacteria in the milk may be wild, as is the case with unpasteurized milk, added from a culture, frozen or freeze dried concentrate of starter bacteria. Rennet is added to cause the casein to precipitate. Rennet contains the enzyme chymosin, which converts k-casein to para-kappa-caseinate (the main component of cheese curd) and glycomacropeptide, which is lost in the cheese whey. After adding the rennet, the

cheese milk is left to form curds over a period of time. The amount of time, and of rennet, varies depending on the variety of cheese being made. Cheese ripening or alternatively cheese maturation, it is responsible for the distinct flavor of cheese, and through the modification of "*ripening agents*." By taking the cheese through a series of maturation stages where temperature and relative humidity are carefully controlled, the cheese-maker allows the surface mould to grow and the mould-ripening of the cheese by fungi to occur. Mould-ripened cheeses ripen faster than hard cheeses, in weeks as opposed to the typical months or even years (Fox &Guinee, 1996). The grading process is one of sampling by sight, smell, taste and texture. Part of the cheesemaker's skill lies in the ability to predict when a cheese will be ready for sale or consumption, as the characteristics of cheese change constantly during maturation (Early, 1995).Cheese is a naturally nutrient-dense food and provides an excellent source of energy and nutrients.The main protein in cheese is casein, with small amounts of other proteins called alpha and beta lactoglobulin. Cheese is a good source of fat-soluble vitamins,Cheeses such as cheddar are good sources of vitamin A. Cheese is also a good source of carbohydrate, fat, minerals and phosphorus. Whether or not cheese's highly saturated fat content actually leads to an increased risk of heart disease. However, some studies claim that cheddar, mozzarella, Swiss and American cheeses can help to prevent tooth decay. Several types of cheese have caused outbreaks of food-poisoning.in maximum case it happens for using unpasteurized milk but some outbreaks of food poisoning due to cheese made with pasteurized milk. Faulty processing such as inadequate heat treatment during pasteurization, mixing with raw milk after pasteurization, or contamination during further processing or distribution of the product is the reason for this incidence(Bone *et al.,* 1989). Variety of microorganisms found for the spoilage and contamination of cheese. These include *Staphylococcusaureus*which produce a toxin that causes illness, *Salmonella* is a major causes of salmonellosis, *Clostridium botulinum* cause Botulism, *Escherichia coli* which indicate that the food was contaminated with pathogenic bacteria. *Listeria, Bacillus cereus* and *Clostridium perfringens*also been found in cheese contamination. Raw milk cannot be guaranteed to be free from pathogenic bacteria (Rampling, 1996). Most of the types of bacteria shown in are liable to be present in some samples of raw milk. A survey of raw milk from farm bulk milk tanks in England and Wales in 1992-3 for *Salmonella* and *Listeria* spp. showed that of 1673 samples, 0.36% contained *Salmonella* spp. and 5.08% contained *Listeria Monocytogenes*(O'Donnell, 1995). The presence of these bacteria may result from direct excretion from the udder or as a result of fecal contamination. In general, if the milk has not been pasteurized it is difficult to ensure the safety of the final cheese no matter how good the control of hygiene during production. Following the major outbreaks of Listeriosis due to contaminated cheese (Linnan*et al.,*1988) many producers of cheese have changed from the use of unpasteurised to pasteurized milk and great improvements have been made in the conditions of hygiene in major cheese factories (Lund, 1990). Microbiological tests on finished cheeses have an important place in quality control, but these tests cannot ensure the microbiological safety of the cheese (Desenclos et al. 1996; Rambling, 1996).

MATERIALS AND METHODS

Sample collection & processing

Cheese samples were collected from two super shops in Dhaka city, SuperShop A and B. Different cheese sample was collected from each super shop. The sampling period was January 2011 to February 2011. Both soft cheese and cream cheese were collected for this study. Soft cheese (sample 2) was collected form supershop B and cream cheese (sample 1) collected from supershop A. Aseptic techniques were follow for the collection and preparation for the samples. All equipments were sterilized before use. Ten-fold serial dilution used for homogenized of the sample. 10gm of cheese was taken into 90ml normal saline. Sterile blender was used to homogenize the sample giving 10^{-1} dilution. Then 1ml from the dilution was transferred to 9ml normal saline giving 10^{-2} dilution and serial dilution was carried out up to 10^{-5}.

Microbiological Analysis

Microbiological analysis for Aerobic plate count, Total coliform count and Total Staphylococcus were performed. Spread plate method were used in this study, 0.1 ml of each dilution was added to the surface of the solidified agar medium. The homogenate samples were inoculated on generalpurpose media, standard plate count media, media for fastidious organisms and media for the selective isolation of bacteria. Nutrient agar media used for aerobic plate (APC), MacConkey for gram negative

bacteria, Mannitol salt agar (MSA) selective for *Staphylococci*,Xylose lysine deoxycholate agar (XLD agar) used in the isolationof*Salmonella* and *Shigella* species, Mannitol Egg Yolk Polymyxin Agar (MYP) for the isolation and identification of *Bacillus* species and pathogenic *Staphylococci*, MFC agar medium was used for the isolation of fecal coliforms, Blood agar are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity, Eosin methylene blue (EMB) is a slightly selective stain for Gram-negative bacteria also provide color indicator for lactose fermenter and Müller-Hinton agar antibiotic susceptibility testing. After inoculation media plates were incubated for 24-48 hours at 37^0C.

Identification of microorganism

Different types of colonies in various culture media were observed. Morphological characteristics including shape, size, color etc of the colonies on different media. Several biochemical tests were performed to identify the bacteria of interest: Catalest test, Triple Sugar Iron (TSI) test, Citrate Utilization test, Motility Indole Urea (MIU) test, Oxidase test and Coagulase test. Confirmatory test for *E.coli*looking for green metallic sheen at EMB agatand for *Staphylococcus aureus*macroscopic clumping with plasmawere performed. Hemolytic reactions were observed.

RESULTS

The log of the CFU/ml values resulting from the analyses of the cheese samples, Enumeration of Aerobic Plate Count (APC) &Total Coliform Count (TCC) present in cheese samples: Total bacteria, *Bacillus* spore, coliform count, *Staphylococcus* counts are listed in table 1.

Table 1. Enumeration of bacteria from cheese sample.

Sample		**Medium**	**Isolate No.**	**Colony Count**	**Cfu/ml**
1	APC	**NA**	**1**	**222**	2.22×10^5
	TCC	MAC	2	1	1×10^2
		MSA	3	1	1×10^6
		RSA	4	1	1×10^6
		BA	5	161	1.6×10^4
			6	Absent	-
			7	Absent	-
			8	1	1×10^6
		XLD	9	Absent	-
		MYP	10	TNTC	-
2	APC	NA	11	345	3.45×10^6
	TCC	MAC	12	136	1.36×10^6
			13	25	2.5×10^7
		MSA	14	2	2×10^6
		BA	15	300	3×10^8
			16	303	3×10^8
		MYP	17	250	2.5×10^4
			18	200	2×10^6
		XLD	19	143	1.4×10^4
			20	21	2.1×10
		RSA	21	Absent	-

Total eighteen isolates were found from two cheese samples. Cultural characteristics of different isolates are listed in the table and figure represents cultural characteristic of several important microbial genera. Gram staining procedure was performed to differentiate into two major group.

Table 2: cultural characteristics and gram reaction of colonies.

Sample	**Media**	**Isolate No.**	**Cultural Characteristics**		**Gram reaction**	**Microscopic observation**
1	MAC	2	Light pink	Small	Gram-negative	Short rod
	MSA	3	Light yellow	Large	Gram-positive	Cocci
	RSA	4	Colorless	Large	Gram-positive	Short rod
	BA	5	Light yellow/Creamy	Large, Non-hemolytic	Gram-positive	Rod
		6	Light yellow/Creamy	Medium, hemolytic	Gram-positive	Rod
		7	Light yellow/Creamy	Medium, Non-hemolytic	Gram-positive	Cocci
		8	Black	Small, hemolytic	Gram-positive	Rod
2	MAC	12	Dark pink	Large	Gram-negative	Rod shape
		13	Colorless	Small	Gram-negative	Cocci
	MSA	14	Yellow	Small	Gram-positive	Cocci
	BA	15	Creamy	Large, Hemolytic	Gram-positive	Rod
		16	Creamy/Off white	Medium, Non-hemolytic	Gram-positive	Rod
	MYP	17	Yellow	Large	Gram-negative	Rod
		18	Pink	Large	Gram-negative	Rod
	XLD	19	Yellow	Small	Gram-negative	Cocci
		20	Colorless	Small	Gram-negative	Rod

Biochemical Tests

Biochemical tests were performed to identify bacterial isolates. Results of biochemical tests were summarized in Table 3.

Table 3: Results of Biochemical Tests.

Isolate No.	TSI				MIU			Citrate	Oxidase	Catalase	Suspected Organism
	Slant	Butt	Gas	H_2S	Motility	Indole	Urase				
2	K	A	+	+	+	+	+	_	_	+	*E.coli*
3	A	K	_	_	_	_	_	_	+	+	*S. aureus*
4	A	A	+	+	+	_	_	_	_	+	Un-identified
5	A	K	_	_	+	_	_	_	+	+	*B. subtilis*
6	K	A	_	_	+	+	_	_	+	+	*B. cereus*
7	K	K	_	_	_	+	+	_	+	+	*S.epidermidis*
8	K	A	_	_	+	+	_	_	+	+	*Listeria* spp.
12	A	A	_	_	+	_	_	_	_	+	*E.coli*
13	A	A	+	_	+	_	_	+	_	+	*Klebsiella*
14	A	A	_	_	+	_	_	_	_	+	*S. aureus*
15	K	A	_	_	+	+	_	_	+	+	*B. cereus*
16	K	K	_	_	_	_	_	_	+	+	*B. subtilis*
17	A	A	_	_	+	_	_	+	_	+	*B. subtilis*
18	K	A	_	_	+	_	_	_	_	+	*B. cereus*
19	K	A	_	_	+	+	_	+	_	+	*E.coli*
20	K	A	_	_	_	+	_	_	_	+	*Shigella*

Note: A= Acid (yellow); K= Alkalaine (red); + = Positive reaction; - = Negative reaction

Table 4: Presence of *E.coli*on EMB agar.

Sample	Isolate No	Green metalic sheen	E. coli Confermation
1	2	Present	Present
2	12	Present	Present

Confirmatory tests for *Staphylococcus aureus*

In this test,two drops of saline were put on to the slide microscopic slide labeled with sample number, Test (T) and control (C). Two saline drops were emulsified with the test organism by using wire loop.

A drop of plasma was placed on the inoculated saline drop corresponding to test and mixed well with loop. The slide was then rocked gently for about 10 seconds. Macroscopic clumping was observed in the plasma within 10 seconds and the result was positive.

Table 5. Confirmatory tests for *Staphylococcus aureus*

Sample	Media	Isolte No	Clot formation
1	MSA	3	Present
2	MSA	14	Present

Antibiotic susceptibility pattern of isolated bacteria

After 24 hours of incubation, Mueller-Hinton agar plates were observed to determine the antibiotic susceptibility pattern of different isolates. Nine isolates were used for antibiotic susceptibility test. Results are summarized in Table 6

Table 6**:**Antibiotic susceptibility pattern of different isolate**.**

Isolate No.	Isolated bacteria	Resistant	Intermediate	Sensitive
2	*E.coli*	AMP, C	-	CN,CRO,CIP
3	*S.aureus*	AMP,CN,CIP,C	-	CRO
5	*B.subtillis*	AMP	-	CN,CRO,CIP,C
6	*B.cereus*	AMP,CRO	CN	CIP,C
7	*S.epidermidis*	AMP	CN,CIP	CRO,C
8	*Listeria*	AMP,CN,CRO,C	CIP	-
12	*E.coli*	-	-	AMP,CN,CRO,C,CIP
15	*B.cereus*	AMP,CRO,CIP	CN	C
16	*B.subtillis*	AMP,CRO,C	-	CN,CIP

Note: AMP = Ampicillin; C = Chloramphenicol; CN = Gentamycin; CIP = Ciprofloxacin; CRO = Ceftriaxone

DISCUSSION

Cheese is a fermented food derived from the milk of various mammals. To make cheese milk is to separate into curds and whey. The curds are used to make cheese. Contaminated cheese has been responsible for outbreaks of food poisoning by several types of bacteria and sporadic cases of illness associated with contaminated cheeses have been reported. Experience shows that there were probably been many others that were undetected or unreported. Some of these bacteria can cause severe illness with long-term consequences and death. The role of this bacterium in foodborne disease was only recognized in the early 1980s, but improvements in methods for the detection and isolation of this organism have led to a progressive increase in the number of outbreaks and sporadic cases of infection detected. In this study, two cheese samples were collected from Supershop-B &Supershop-A of Dhaka city. In the present investigation, it was noticed that cheeses were contaminated with huge load of microbes. Samples collected from Supershop-B market had high bacterial load (3.45×10^6cfu/ml) than those from Supershop-A market (2.22×10^5 cfu/ml). Total coliform count in cheese sample collected from Supershop-B market had huge microbial load (2.5×10^7cfu/ml) than the sample collected from Supershop-A market (1×10^2cfu/ml). Different types of media were used to investigate the pathogenic organisms present in cheese. Such as Nutrient agar (NA), MacConkey agar

(MAC), Mannitol salt agar (MSA), Xylose lysine deoxycholate agar (XLD agar), Mannitol egg yolk polymyxin agar (MYP), MFC agar, Rogosa SL agar (RSA) and Eosine-Methylene blue agar (EMB). Suspected organisms found in cheese sample collected from Supershop-A market were *E.coli, S.aureus, B.subtillis, B.cereus, S.epidermidis* and *Listeria* sp. And the sample that is collected from Supershop-B market, pathogenic organisms found were *Klebsiella, S.aureus, B.cereus, B.subtillis, E.coli*and *Shigella.* Among these *Listeria* is the most consistently pathogenic species causing listeriosis and *Listeria* infections are acquired primarily through the consumption of contaminated foods, including soft cheese. The Kirby-Bauer disk diffusion test was used in the experiment to determine the antibiotic susceptibility pattern of isolates.In this test, the standardized bacterial isolate is spread on an agar plate and then disc containing specific concentration of antibiotics were placed and incubated at 37^{o}C overnight. After 24 hour incubation, the agar plates were observed. *E.coli*was found to be sensitive against Gentamycin, Ceftriaxone, and Ciprofloxacin, and resistance against Ampicillin and Chloramphenicol. *Listeria* were resistance against Ampicillin, Gentamycin, Chloramphenicol and Ceftriaxone. *Bacillus* spp . those are isolated from cheese almost sensitive against Chloramphenicol and Ciprofloxacin, and resistance against Ampicillin and Ceftriaxone.

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