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Isolation of Pathogens From Dairy Products And Effect of Temperature on Pathogens

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ABSTRACT

Since times immemorial, microorganisms have been playing both useful and harmful roles in human life and this has led to the need of studying these microscopic biological agents extensively. Milk being a nutritious food for human being also provides an ideal environment for microbial growth. Milk and milk products e.g. milk powder, milk, cheese, curd and ice cream etc. constitute important nutritional components for all age groups and also nutritive for pathogens. Study was carried out by using various microbiological techniques to isolate and identify pathogens. 18 samples of milk and milk products i,e. Milk, Shrikhand, Amrakhand, Curd, Chocobar, Ghee, Milk powder, Ice cream were analyzed for pathogen and effect of temperature on pathogens isolated from milk and milk products. In this study it can be concluded that this milk products poses a serious health risks.

Keywords : Temperature, Pathogens, Dairy products and Microorganisms.

INTRODUCTION:

Control of food borne pathogens and prevention of related diseases are major tasks that public health will face in the 22nd century. More than 200 known diseases are transmitted through food by a variety of agents that include bacteria, fungi, viruses, and parasites. According to public health and food safety experts, each year millions of illnesses in the USA and throughout the world can be traced to food borne pathogens. While the food supply in the United States is one of the safest in the world, the Center for Disease Control and Prevention estimates that 76 million people get sick, more than 300,000 are hospitalized, and 5,000 Americans die each year from food borne illness. The risk of food borne illness has increased markedly over the last 20 years, with nearly a quarter of the population at higher risk for illness today. Consequently, preventing illness and death associated with food borne pathogens remains a major public health challenge. Should the dairy industry be concerned about food safety? The answer is yes, and there are several good reasons why such as: Bulk tank milk contains several food borne pathogens that cause human disease, outbreaks of disease in humans have been traced to the consumption of raw unpasteurized milk and have also been traced back to pasteurized milk, raw unpasteurized milk is consumed directly by dairy producers and their families, farm employees and their families, neighbors, etc., raw unpasteurized milk is consumed directly by a much larger segment of the population via consumption of several types of cheeses including ethnic cheeses manufactured from unpasteurized raw milk, entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms and subsequent contamination of processed food products, pasteurization may not destroy all foodborne pathogens in milk, and faulty pasteurization will not destroy all foodborne Pathogens. A logical approach to control foodborne pathogens in dairy farms should be to define areas in the dairy farm that serve as foodborne pathogens reservoirs and management practices that contribute to the persistence and spread of pathogens from these reservoirs. The number and types of microorganisms present in milk and dairy products depends on the microbial quality of milk used, heat treatment of milk, the conditions in which the products are manufactured, the temperatures and duration of storage, feeding of the animals, season, area, general sanitation in the plant, quality of starter cultures, occurrence of phages, quality of rinsing water, etc. Examination for the presence and number of specific micro-organisms is, therefore, an integral part of any quality control or quality assurance plan and it may be applied to a number of areas: raw materials, intermediate samples, finished product, or environmental equipment sites.

The extent of contamination and subsequent microbial multiplication directly determine the microbiological quality of the product hence the major emphasis for combating the harmful effect of microorganisms in dairy products includes the following measures: Avoiding or minimizing contamination of milk, refrigerated storage of raw milk before processing, avoiding prolonged storage of processed milk and milk products under favourable ambient conditions to control microbial multiplication.

The Milk when secreted in the healthy udder is almost sterile. It is only during subsequent milking and post milking operations that raw milk gets various microorganisms due to contaminations from the surrounding fresh milk obtained from healthy udder under sanitary conditions contains relatively few contaminated by man, his practices and the environment (International Dairy Federation, 1974) unpasteurized milk contain a wide range of bacteria, principally those causing mastitis and also the faecal flora arising from udder contamination.

A broad spectrum of microbial pathogens contaminates human food and water supplies and cause illness after they or their toxins are consumed. These include a variety of enteric bacteria, aerobes and anaerobes, viral pathogens and yeasts. During past decades microorganisms such as staphylococcus spp. Salmonella spp. were reported as the most com-

mon food borne pathogens that are present in many foods and able to survive in milk and fermented milk products (Alm, 1983; Ahmed et. al .,1986; Ryser and Marth ,1988 ; Schaak and Marth ,1988; Cangnella et. al., 1998; Dineen et. al., 1998 ; Gulmz and Guven, 2003; Tekinsons and ozdemir,2006).

Escherichia coli is responsible for several out breaks of diarrhea in children and adults after ingestion of contaminated milk and dairy products different studies showed that 1-5% of food borne infections were related to consumption of milk and dairy products that 53% of cases of food borne infections caused by contaminated cheese and that enteropathogenic Escherichia coli is the causative agent of 18.33% of these cases. Examination of the presence of Escherichia coli as an indicator of faecal contamination or poor hygienic practices has traditionally been done in dairy plants. It is well known that some strains might be enteropathogenic or enterotoxigenic. Both of these groups have been responsible for outbreaks of diseases involving cheese and milk (Anonim, 1994).

MATERIAL AND METHODS:

Collection of dairy products i,e. milk packets, milk powder, curd ,ghee, chocobar, shrikhand, amrakhand, ice cream, etc. and 18 Samples of dairy products like Milk packet, Milk powder, Shrikhand, Amrakhand, Ice cream, Curd and Chocobar were done from the market Packed dairy food samples were directly transported to the laboratory in ice box. They were stored in refrigerator and analyzed within 24 hours. Out of 18 samples 5, 3, 2, 2, 2,1,1,2 packed food samples were milk, milk powder, amrakhand, shrikhand, ice cream, ghee, chocobar, curd sample respectively. Isolation of the pathogens i.e. Salmonella, Staphylococcus, Escherichia coli from dairy samples on selective media petriplates.

Microbiological analysis:

A portion (1 g or 1 ml) from each sample was taken aseptically and diluted in 9 ml sterile distilled water the diluted sample was streak inoculated on sterile selective media as given below

1. Eosine Methylene Blue (EMB) for Escherichia coli
2. Mannitol Salt Agar (MSA) for Staphylococcus aureus
3. Wilson's and Blair (W& B) for Salmonella typhi Inoculated petriplates were incubated at 37^0C for 24hrs. Identification of the pathogen on the basis of cultural characteristics, Gram staining, Biochemical test, Sugar fermentation test, Catalse test, Oxidase test, Urease test and Coagulase test.

Identification of pathogens:

1. Cultural characteristics, gram nature and colour of colonies were noted.
2. Biochemical examination colonies from each petriplate were picked, subcultured, incubated at 37^0C and then identified by the various biochemical tests.

Biochemical tests were performed to confirm Escherichia coli, Staphylococcus aureus and Salmonella typhi using Catalase test, Indole test, Methyl red test, Voges prousker test, Urease production, Citrate utilization test and glucose, lactose, mannitol, sucrose sugar fermentation test (Khalil Ahmed, et.al., 2009, Ashaman Ali and Warda S. Abdelgadir, 2011).

Effect of temperature on pathogens of dairy pack food:

Temperature is the necessary environmental factor for the growth of microorganism. Different microorganisms grow at different temperature.

Effect of temperature on Pathogens:

- Samples collected from market in aseptic condition and transfered to laboratory in an ice bag.
- Samples kept at different temperatures in laboratory at 37^0C, 42^0C, 47^0C, 52^0C, 62^0C, 67^0C,72^0C, 77^0C for 24 hrs.
- Sterilized selective media plates were prepared for EMB, MSA, W&B, Monsur's, media Petriplates.
- Samples were streak on petriplates containing selective media and kept the Petriplates in incubator for the incubation at 37^0C for 24hrs.
- After 24hrs the results were observed.

Results:

A. Morphological,gram and cultural characteristics of Escherichia coli,Salmonella

typhi,Staphylococcus aureus were studied and results are given in **Table- 1**

B. Biochemical test for identification of Escherichia coli,Salmonella typhi,Staphylococcus

aureus were performed and results are given in **Table-2**

Table: 1.Morphological and culture characteristics of isolated pathogens.

Sr.No	Isolated pathogen	Gram staining and morphology	Culture characteristics on selective media
1	Escherichia coli	Gram –ve coccobacilli	Colonies showing green metallic sheen.
2	Staphylococcus aureus	Gram +ve cocci	MSA medium turns red to yellow
3	Salmonella typhi	Gram –ve rod shaped	Black colonies on wilsons and blair medium.

Table 2.Biochemical tests

Sr.no	Tests	Dairy pack food sample's																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		A	S	A	A	A	N	S	S	S	A	`A	S	S	S	S	N	N	N
1	Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	MR	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-
3	VP	+	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-
4	Citrate	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-
5	Mannitol	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-
6	Maltose	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-
7	Sucrose	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-
8	Glucose	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-
9	Lactose	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-
10	Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sr.no	Tests	Dairy pack food sample's																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		A	S	A	A	A	N	S	S	S	A	`A	S	S	S	S	N	N	N
11	Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	Coagulase	+	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-

A=Staphylococcus aureus
t=Salmonella typhi
N= No growth

Table 3. Effect of temperature on pathogen.

Sr. no	Pathogen	Temperature								
		37^{0}C	42^{0}C	47^{0}C	52^{0}C	57^{0}C	62^{0}C	67^{0}C	72^{0}C	77^{0}C
1	Escherichia coli	-	-	-	-	-	-	-	-	-
2	Salmonella typhi	+	+	+	+	-	-	-	-	-
3	Staphylococcus aureus	+	+	+	-	-	-	-	-	-

Table: 4. Distribution of various packed food samples on the basis of pathogens.

Sr.No.	Type of Sample	Total no.of samples	Number of samples showing presence of pathogen		
			Escherichia coli	Salmonella typhi	Staphylococcus aureus
1	Milk packet	5	-	3	1
2	Milk powder	3	-	-	-
3	Amrakhand	2	-	-	1
4	Shrikhand	2	-	-	1
5	Ice cream	2	-	-	1
6	Ghee	1	-	-	-
7	Chocobar	1	-	-	1
8	Curd	2	-	-	1

Discussion:

In milk industry presence of total and faecal coliform indicates pollution of faecal origin or a contamination due to technological or hygienic failure (Barthe et. al, 1998) when they are numerous. They may lead to food poisoning (Auedigier et. al., 1980). Moreover the presence of facal coliforms usually indicates recent faecal contamination, because these bacteria cannot survive apart from the intestine for a long time and their number is generally proportional to pollution degree produced by faeces (Goursaud, 1985). Milk constitutes a favorable medium to development a staphylococcus aureus which enterotoxin responsible of food poisoning acts quickly at room temperature (Bourgeois et. al., 1996). The presence of salmonella spp. may pose a great risk for public health since salmonella outbreaks from ice cream have been reported previously (Vought et. al., 1998). In the present study the results obtained from the microbial analysis of milk and milk products viz. milk packets, milk powder, Amrakhand, Shrikhand, Ice cream, Ghee, Chocobar and Curd showed that milk and milk products were contaminated with microorganisms of public health concern. Table no.1 shows morphological and culture characteristics of isolated pathogens. Table no.2 shows biochemical tests, sugar fermentation test, oxidase test, catalase test, Urease test, Coagulase test etc. of isolated pathogens. Table no.3 shows effect of temperature on the pathogens. The distribution of various packed milk food samples on the basis of presence of pathogens is given in Table 4. It was found that Out of 5 milk packets samples, 1 sample was contaminated with Staphylococcus aureus and 3 samples were contaminated with Salmonella typhi. Out of 3 milk powder samples, there was no any contamination found. Out of 2 amrakhand samples, 1 sample was contaminated with Staphylococcus aureus. Out of 2 shrikhand samples, 1 sample was contaminated with Staphylococcus aureus. Out of 2 ice creams samples 1 sample was contaminated with Staphylococcus aureus and 1ghee sample there was no any contamination found. In chocobar sample 1 sample was contaminated with Staphylococcus aureus. Out of 2 curd samples sample 1 sample was contaminated with Staphylococcus aureus. All above pathogens killed at 55^{0}C-70^{0}C temperature in laboratory and the pasteurization temperature was above but there was contamination in milk and milk products. All these results can be explained by the fact that the method of production of the various traditional foods are usually primitive compared to modern way of food preparation and the major risk enhancing factors are the use of contaminated raw materials, lack of pasteurization, use of poorly controlled natural fermentation , inadequate storage and maturation conditions.

Summary:

S. aureus and E. coli showed growth at 37^{0}C, 42^{0}C and 47^{0}C temperature and S. typhi showed growth at the temperature 37^{0}C, 47^{0}C, and 57^{0}C. Temperature above 60^{0}C kills all pathogens present in milk and in dairy industries. Temperature above 77^{0}C which is sufficient to kill the pathogen. This is very practical consideration since milk is preserved by employing low temperatures to prevent changes due to microbial activity and by high temperatures to reduce microbial population and destroy pathogens. The contamination takes place after pasteurization from equipment, cans, bottles and water.

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