

Bacteriological Assessment Of Paneer Sold In Guwahati, Assam

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Abstract: Paneer is one such product which is a regular dietary favourite among the Indians. So the present study was conducted to assess the bacteriological quality of Paneer sold in Guwahati city, Assam. A total of 30 samples were collected from various areas of Guwahati city. All samples were analysed for total viable count of pathogenic bacteria on different solid agar media and their antibiotic susceptibility pattern. All the 30 samples had bacteriological counts ranging from 1×10^2 to 3.0×10^7 CFU/gm. Of all the samples *Staphylococcus spp.* was found in 100% of the samples, *Escherichia coli* in 66.7%, *K. pneumoniae* in 40% and *S. typhi* in 13.3% of the samples. The heavy bacterial contamination seen in all samples predominantly in *Staphylococcus spp.* and fecal coliforms can be attributed to poor hygienic conditions during paneer preparation, handling and storage. The study suggests the need for more strict preventive and control measures to avoid pre and post process contamination in milk food products.

Index Terms : Paneer, Milk product, Bacterial contamination, *Staphylococcus aureus*, MRSA

1. INTRODUCTION

Milk, being a complex mixture, nutritious, with a high level of water and a pH close to neutral, is highly perishable. It is a product highly conducive to microbial growth, especially bacterial pathogens (Adams, 2000). Depending on the manipulations it is subject to, milk can have its physical, chemical and biological properties easily altered by the actions of microorganisms. Dairy products are defined as food produced from milk. They are usually high energy-yielding food products. Dairy products are mostly found in European, Middle Eastern, Indian cuisine, aside from Mongolians cuisine. They are little known in traditional East Asian cuisine (Grandsen ER, 1997).

Different foods implicated in foodborne outbreaks in India are milk and milk products such as dahi, khoa, butter milk, sweets, kheer; meat, poultry, fish, fowl, sea food such as prawns; cooked and uncooked rice; samosa, batatawada, tamarind, and cooked as well as uncooked vegetables. During past decades, microorganisms such as *Staphylococcus aureus*, *Salmonella spp.*, *Escherichia coli* O157:H7, *Shigella spp.*, *Listeria monocytogenes* and *Yersinia enterocolitica*, were reported as the most common food borne pathogens that are present in many foods and able to survive in milk and fermented milk products (CD alert, 2017).

1.1. Objective : As dairy products are potential source of pathogenic microorganisms, our present study was undertaken to assess the bacteriological quality of paneer samples collected from local dairies of Guwahati, Assam.

2. MATERIAL AND METHODS

2.1. Collection of sample:

30 different dairy samples of paneer were collected from 30 different local dairies and shops (5 samples from each dairy industry) of different localities of Guwahati, Assam. All samples were collected using sterile polythene bags and analyzed at Department of Microbiology, Sri Sankaradeva Nethralaya, Guwahati, Assam.

All the samples were processed aseptically as described under. The bags were swabbed with 70% ethanol before opening. 1 g of each sample was added to 9ml of the sterilized ringer's solution to prepare 10^{-1} dilution. Further 10^{-2} and 10^{-3} dilutions were prepared. 1ml of each dilution was plated in duplicate on the solid media (Hi-Media, Mumbai) - Mannitol Salt Agar, MacConkey Agar (MCA), and Deoxycholate Citrate Agar (DCA). The dilution was spread with the help of sterilized spreader and incubated at 37°C for 24 to 48 hours. After 24-48 hours of incubation colonies of pathogenic bacteria were counted and expressed in Colony Forming Units/g (CFU/g) (Robinson RK, 1981).

2.2. Identification and Antibiotic sensitivity testing of Pathogens:

A maximum of five discrete colonies from each plate showing microbial growth were picked for further identification. Identification was performed as per standard methods described, based on - colony morphology, Gram's reaction, biochemical test (Collee JG et al., 2006). Identification of Gram negative bacilli were performed on the Vitek 2 - Compact, Biomerieux.

The antibiotic sensitivity was done by standard Disk diffusion method as per CLSI guidelines (CLSI, 2017). An inoculum prepared in sterile peptone water by direct colony suspension, equivalent to a 0.5 McFarland standard, was spread on Mueller hinton agar (MHA). The antibiotic disc with known disc potency were procured from Hi Media, Mumbai. The inoculated plates were incubated at 37°C overnight. A clear zone of inhibition indicated the sensitivity of isolates to the antibiotic. The *Staphylococcus aureus* isolates were tested for Gentamicin (10 mcg), Vancomycin (10mcg), Streptomycin (10mcg), Ciprofloxacin (10 mcg), Cefoxitin (30mcg). Members of Enterobacteriaceae (*E. coli*, *K. pneumoniae*, *Salmonella typhi*), were tested against Gentamicin (10mcg), Cephalexin (10mcg), ciprofloxacin (30mcg), Ceftazidime (30mcg), Ampicillin (10mcg), Chloramphenicol (5mcg), tetracycline (30mcg).

3. RESULTS AND DISCUSSION

Our findings reveal that all the samples were contaminated with one or more than one pathogens (Table 3.1). *Staphylococcus aureus* (30) contamination was commonest followed by *Escherichia coli* -20(66.7%), *Klebsiella pneumoniae* -12(40%) and *Salmonella typhi* -

4(13.3%)(Table 3.2). 80% of samples were found to be contaminated with more than one pathogen – 40% each with 3 and 2 pathogens and 20 % single pathogen. The antibiotic sensitivity pattern of the isolates is shown in Table 3.3.

In majority of samples the count of individual pathogens varied from 100 to 3000000 c.f.u/g of the samples but in some samples the pathogens were found in considerably high numbers. Sachdeva and Singh (1990) observed the microbiological characteristics of paneer stored at 8–10 °C and reported that total plate count related well with its spoilage. The fresh paneer samples showed that the initial count ranged from 2.3×10^4 to 9.0×10^4 cfu/g. The total plate count of the spoiled samples ranged from 1.58×10^6 to 4.5×10^7 cfu/g (Sachdeva S et al., 1988).

Table3.1: Bacterial pathogens in paneer samples (n=30) along with colony count

Sample no.	Bacteria Isolated (Colony forming units/g)			
	<i>S.aureus</i> Cfu/g	<i>K.pnumoniae</i> Cfu/g	<i>E.coli</i> Cfu/g	<i>S.typhi</i> Cfu/g
1	2.9×10^3	-	5.0×10^3	2.4×10^2
2	4.0×10^3	-	1.3×10^3	-
3	1.7×10^3	2.0×10^3	-	1.0×10^2
4	1.5×10^3	-	-	-
5	2.2×10^3	-	1.1×10^3	1.0×10^2
6	1.4×10^3	-	1.6×10^3	-
7	2.5×10^3	7.0×10^3	-	-
8	6.0×10^3	-	-	-
9	5.0×10^2	-	2.0×10^3	2.0×10^2
10	1.0×10^3	-	-	-
11	6.0×10^5	-	5.0×10^3	-
12	4.0×10^6	3.0×10^6	3.0×10^7	-
13	1.7×10^3	-	-	-
14	1.5×10^3	5.0×10^3	5.0×10^5	-
15	2.2×10^3	-	-	-
16	1.4×10^3	2.0×10^4	4.0×10^6	-
17	2.5×10^3	-	1.3×10^3	-
18	6.0×10^3	-	6.0×10^3	-
19	5.0×10^2	6.0×10^3	-	-
20	1.0×10^3	4.0×10^3	6.0×10^3	-
21	2.5×10^3	-	5.0×10^3	-
22	1.4×10^3	2.0×10^3	1.5×10^3	-
23	3.5×10^3	-	2.0×10^4	-
24	6.0×10^3	-	-	-
25	3.0×10^2	3.0×10^5	5.0×10^6	--
26	1.0×10^3	-	4.5×10^3	-
27	5.0×10^5	4.0×10^4	-	-
28	4.0×10^5	2.0×10^6	1.4×10^2	-
29	1.7×10^6	-	3.0×10^3	---
30	1.5×10^3	5.0×10^3	2.0×10^4	-

Table 3.2: Total number and percentage of samples positive for each pathogen

	Number of samples positive for pathogens (n=10)	Number of positive individual pathogens			
		<i>S.aureus</i>	<i>K.pnumoniae</i>	<i>E.coli</i>	<i>S.typhi</i>
No.of positive samples	30	30	12	20	4
Percentage (%)	100	100	40	66.7	13.3

Table 3.3: Antibiotic susceptibility pattern of the pathogenic isolates

Organism	Antibiotic sensitivity (%) / Total No. isolates	G	S	Va	Cf	Caz	Am	Ctx	C	Cx
<i>Staph.aureus</i>	30	100	80	100	80	-	80	-	80	80
<i>E.coli</i>	20	100	100	-	100	100	75	100	100	-
<i>K.pneumoniae</i>	12	100	100	-	100	100	50	100	100	-
<i>S.typhi</i>	4	100	-	-	50	-	100	100	100	-

Table 3.3 : G-Gentamicin , Va- vancomycin, Cf-ciprofloxacin, Caz- Ceftazidime, Am- ampicillin, C- chloramphenicol, streptomycin, Cx - Cefoxitin,

Coliforms can multiply at room temperature and dairy products are good media for bacterial growth.. In our study 80% of paneer samples the coliform count was above the acceptable standards which is similar to the findings reported in other studies(Sachdeva S et al,1988; Vaishnavi et al. 2001; Cannon RY ,1966; Sunga FC et al. ,1966).

S.typhi contamination is probably due to handling by asymptomatic *S.typhi* carriers. In a study on food handlers, 16.66% of food handlers were found to be asymptomatic *S.typhi* carrier (Senthilkumar B et al.2005).

Staphylococcus aureus was cultured from all the specimens. Among the *S.aureus* isolates, 20% (6/30) were methicillin resistant. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacteria that is multidrug resistant. MRSA most often causes skin infections in the general community. It may circulate in the community through asymptomatic carriers. MRSA is now endemic in India and reports suggest that community acquired MRSA is increasing in numbers(Satish G et al.). *Staphylococcus aureus* may come from the wound on the hands of the worker that handle the product, nasal canal or other parts of the body. Enteric pathogens may find access from faecal matter if hands are not properly washed or through water if it is contaminated with sewage or faecal matter (APHA, 1992).

Presence and multiplication of pathogenic microorganisms pose a serious health problem to consumers if proper hygienic conditions are not followed at time of manufacturing and packaging and subsequently proper storage conditions are not followed. Most of pathogens are destroyed during pasteurization except *Bacillus cereus*, *Clostridium* spp (Joshi S et al.,2013).

The source of most of the paneer consumed by Guwahati city consumers is from the unorganised sector. The dairy farms located in the outskirts of the city are main bulk suppliers. The milk is delivered in large cans to the sweet makers. In most of the paneer manufacturing units paneer is manufactured in bulk quantities. The paneer being sold is usually at room temperature overnight immersed in water. According to Bureau of Indian Standards (IS:10484-1983) paneer is prepared by the combined action of acid coagulation and heat treatment of buffalo or cow milk or a combination of both. The probable source of contamination are environmental sources like water , air , equipments , packaging material and handlers. According to CDC update on outbreak related illnesses and hospitalizations following contaminated dairy consumption in united States, contaminated unpasteurized milk and milk products like cheese caused 96% of illnesses out of 760 illnesses per year , thus causing 840 times more illnesses than pasteurized products(Solenne C et al.,2017). The number of bacteria in milk, directly influences the quality and safety of dairy products (Grandson et al).

The application of HACCP (Hazard Analysis and Critical Control Points) System were made in dairy industry in order to maintain high quality levels in dairy manufacturing and production of foods for safe consumption, for increase the requirements for rapid and more automated microbiological techniques (Microbiology and food safety committee, NFPA, Washington DC).

The Food products Standards and Food Additives (FSSAI) has notified the FSS (Prohibition and Restrictions on sales) Amendment Regulations, 2017 regarding revised standards for milk and milk products (Gazette notification milk products 2017). (Table 3.4)

Table 3.4: Microbiological standards for Paneer , (Revised Standards) FSSAI

no	characteristics	requirement
	bacterial count, per gram	1,000
	coliform count, absent in	1 g
	yeast and mould count absent in	1 g

The present study highlights heavy contamination of paneer samples with organisms like *Staphylococcus aureus* , *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*. This indicates the poor hygienic conditions and faults in manufacturing/handling during the process of preparation. There is a basic lack of knowledge about hygiene and the hazards of food contamination among the producers as well as the consumers. In Conclusion, we recommend that dairy products like paneer, manufactured by unorganised sector needs to be brought under the regulations for commercial sale of these products.

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