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Incidence of Toxin Producing Bacteria in Milk and Milk Products

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ABSTRACT

Milk and milk products is a very good source to support the growth of microorganism. Their presence may cause health hazards. Considering its unhygienic aspects in view, the present study was undertaken to judge the occurrence of toxin producing microorganism. A good number of milk, curd, ice-cream and khoa samples supplied to Tura town (Head quarter of District West Garo Hills, Meghalaya) were assessed for their total bacterial, clostridial and staphylococal counts. Out of total clostridia and total coagulase positive staphylococcus count about twenty percent were identified as clostridium botulinam and ninety percent as staphylococcus aureus respectively. These isolates, pure and identified cultures of these organisms were incubated in litmus milk for one week for production of toxins. Heat stability of toxins were determined by heating the inoculates at 75, 85, and 100.c for five minutes. Toxicity of toxins produced by these microorganisms was tested by intra peritoneal injection and orally feeding to mice and seen that 1.5 c.c intra peritoneal injection was lethal dose. Heating of inoculates at 85.c for five minutes has partially inactivated botulinam toxin, whereas it has not effected staphylococcus enterotoxin. Heating at 100.c for five minutes has completely inactivated botulinam and partially inactivated staphylococcal enterotoxin. It can be concluded from the experimental results that the bacteriological quality of milk and milk products supplied to Tura town is very poor.

Keywords : Toxin Producing Bacteria, Milk, Dairy Products.

I. INTRODUCTION

Milk and milk products is a suitable food, support vigorous multiplication of a number of microorganisms. The survival and presence of these micro-organisms may spread epidemic diseases through them, if these are produced and handled under unhygienic conditions. Clostridium botulinum and stablyococcus aureus is a versatile pathogens in human and animals which is responsible for many diseases raging from skin infection to life threatening. (Genigeorgis, 1989) They are also known as one of the most important agent of food poisoning worldwide, because of their extracellular metabolities production and their wide occurrence in soil, air, water, human body and even over clothes. These toxinogenic organisms may gain access to raw milk and survive in milk and milk products if these are not properly pasteurized, or get recontaminated with raw milk. The outbreaks of this food poisoning are exposing serious challenges to the workers engaged in the field of food/dairy microbiology (Janga and Singh2010). Unavailability of organized dairy sector in the region may be the reason of unhygienic milk production in the area, thus the present investigation was undertaken to assess the incidence of toxin producing bacteria in milk and milk products in Tura town of Garo Hills regions of Meghalaya.

II. METHODS AND MATERIAL

(A) Collection, Handling and Treatment of Samples:

(i) **Milk –** under the present study a total of 30 milk samples were collected from different selling booths, vendors, dairy farms and sweet shops. The samples were collected aseptically in clean sterilized glass bottles and cooled promptly to about $4^{\cdot C}$ in an ice-box to minimize further multiplication of bacteria. The samples immediately after arrival at laboratory were subjected to bacteriological analysis as per Bureau of Indian Standard (B.I.S), 1981.

(ii) **Dahl/Curd –** Twenty dahi samples were collected from sweet shops and vendors. The samples were collected aseptically in clean sterilized bottles and cooled to $4^{\cdot C}$ in an ice-box. For the analysis of dahi 11 gm. of sample was

mixed in sterilized glass mixer for 2 minutes at 3,000 rpm with 99 ml of buffered dilution. Suitable dilutions (1 and 2 for Clostridia and Staphylococci counts were plated for their respective bacterial counts.

(iii) **Ice-Cream -** A total of 12 ice- cream samples from local market were collected in the same manner as for dahi samples. The samples were analyzed for their microbial quality as per the BIS, 1981.

(iv) **Khoa -** Ten Khoa samples were collected from different sweet makers of the Tura town. The samples were hold at about $4^{\cdot C}$ in order to prevent the further growth of micro-organism. For the analysis of the Khoa sample suspension was prepared by transferring 11 gm. of sample with the help of a sterile spatula to 99 ml buffered dilution blank in sterile blender and was shake vigorously for 2-3 minutes. The suspension thus obtained was uniform (Sharma et al 1972). The suitable dilution was plated for their counts.

The clostrisel agar medium was used for enumeration of clostridium, while for staphylococcus count was done as per methods described by Chalmers, 1962.In order to confirm the presence of either *staphylococcus aurious* or *clostridium botulinum* five isolates from each product were studied in their respective selective media as prescribed by BBL manual.

Identification of Colonial Isolates: Five colonies resembling in their colony characteristics to clostridium and staphylococci were picked up from each product. The colonies were then transferred to the sterilized litmus milk tubes and grown in anaerobic conditions; the tubes were then incubated at $30^{\cdot C}$ for 48 $\pm$ 2 hours for *clostridium botulinum*. While for staphylococcus colonies from Vogel and Johnson agar plates incubated at 37'c for 48 $\pm$ 2 hours.

In general, all the biochemical tests were performed and interpreted as per procedure recommended in 'Identification Methods for Microbiologist' by Gibbs and Skinner (1966), Bergey's manual of determinative bacteriology by Breed et al (1957) and BBL manual (Table 1).

Table 1: Diagnostic scheme for identifying *Clostridium botulinum* and *staphylococcus aureus*

S.No.	Name of Test	Clostridium botulinum	Staphylococcus aurious
1.	Glucose	+ ve	+ ve
2.	Lactose	- ve	+ ve
3.	Sucrose	- ve	+ ve
4.	Maltose	+ ve	+ ve
5.	Fructose	+ ve	not performed
6.	Manitol	- ve	+ ve
7.	Gelatin	+ ve	+ ve
8.	Nitrite	- ve	+ ve
9.	Indole	- ve	not performed
10.	Coagulase production	not performed	+ ve

Note: Out of the 20 isolated from all the products six were found to be resembling *clostridium botulinum* and nine were resembling *stablylococcus aureus.*

Production of Toxin

Isolated, pure and identified cultures of these organisms were incubated in litmus milk for one week for the production of toxin.

Determination of Toxicity

The subject selected for testing the toxicity of these two organisms were mice. All the animals ranged in their age group of 1-2 months. In all, 10 mice were used in this experiment. For testing the toxicity of both organism 1 ml,1.5 ml and 2 ml of pure inoculates were injected intraparetoneally and also orally in three different subjects and symptoms were recorded.

Heat treatment of Toxins

The inoculates were heated at 75, 85 and $100^{\cdot C}$ for 5 minutes and were injected in the quantity of 1.5 ml as was to be lethal. The toxicity symptoms were noted.

III. RESULT AND DISCUSSION

In the present study the details of the occurrence of total *clostridial* and *stabhylococci* count in milk and milk products have been investigated. An attempt has also been made to isolate the *clostridium botulinum* and *staphylococcus aureus* species from the respective groups. The isolated species were then incubated in litmus milk for a week for the production of their toxins.

Later on these toxins were tested for their toxicity and heat stability by the use of laboratory mice.

Thirty milk samples, 20 dahi samples, 12 ice-cream and 10 samples of khoa supplied to Tura town were assessed for their *clostridia* and *staphylococci* count per ml/gm.

As evident from the table 2 the *clostridial* count was recorded maximum from dahi (246.95 ± 19.49 per gm) that was nearer to khoa (195.5 ± 35.52 per gm) followed by ice-cream and milk (111.33 ± 33.67 and 81.13 ± 9.79 respectively).

Khoa possessed the highest *staphylococci count* (273.70 ± 54.82 x 10^2 per gm) that was nearer to the count of dahi 237.2 ± 19.77 x 10^2 per ml) followed by milk (222.7 ± 20.68 x10^2 per ml) and ice-cream (95.45 ± 18.65 x 10^2 per ml) respectively.

Out of total *clostridia* counted about 20 percent were identified as as *botulinum* species while cogulase positive *stabhylococcus aureus* were identified as 90 – 95 percent.

Table 2: The minimum maximum and average of clostridia and stabhylococci count per ml/gm in milk and milk products:

S. No.	Name of Product	Clostridia Count per ml/gm			Staphylococci per ml/gm		
		minimum	maximum	average	minimum	maximum	average
1.	Milk	15	181	81.133±9.79	59	508.5	222.7±20.68
2	Dahi	92	410	246.95±19.49	94	420.5	237.2±19.77
3	Ice-cream	12	355	111.33±33.68	25	255	95.45±18.65
4	Khoa	37	380	195.5±35.52	100.50	651	273.70±54.82

Just before toxicity tests the *clostridium botulinum* and *staphylococcus aureus* were counted as 45x10^6 per ml, respectively. In order to determine the lethal dose of these toxins 2, 1.5 and 1 cc of inoculate were intraparetoneally injected and 2 and 1 c.c. orally fed to mice. Two and 1.5 cc intraparetoneal injections of each inoculate resulted in death of mice whereas 1 cc did not show any effects. Oral feeding slightly effected at 2 cc concentration whereas failed to show any effects at 1 cc concentration in both cases (Table 3 and 4). It was assured that their lethal doses were 1.5 cc intraparetoneal injections. Heating of inoculates at $85^{\cdot C}$ for 5 minutes has completely inactivated botulinal toxin where as it has partially inactivated *stabhylocccal enterotoxin*.The finding of this study have also been supported by Neill and Grimes (1947) they fouind that 33% of the ice-cream in cork city contained clostridia. The range of *stabhylococci* noted 25x$10^{2\ to}$ 255x$10^{\ 2}$ per ml of ice-cream. This higher count may be due to the higher *stabhylococci* count present in milk used for ice-cream. In contrast to our finding, Faroane (1966) reported the occurance of *staphylococci* in ice-cream as very low, whereas Pogeria and Saraswat (1969) and Singh et al (1974) reported *staphylococci* in much higher number. A number of workers Kudthodkar and Singh (1964) have reported the presence of *stabhylococci* in Khoa obtained from various cities. The numbers of these organisms in their studies have always been higher than in our study.

In contrast to *clostridium botulinum* the number of staphylococcus aureus to produce lethal dose of toxin for mice, was much more in the present study (69x$10^{6)}$. Other workers (Donnelly etal 1969) reported that a concentration of 5x10^7 *staphylococcus aureus* cell per ml was required before enterotoxin was detected. By the injection of 1.5 cc of inoculates the toxicity symptoms developed after $4^{1/2}$ hours in case of *clostridium* and after $6^{1/2}$ hours in case of *staphylococci.* In case of clostridium specific symptoms were noted as diarrhoea, vomiting, nausea and paralysis, where as in case of *staphylococci* the symptoms were vomiting, diarrhoea and prostration etc.

As against our observation Ayres et al (1969) have indicated that *clostridium botulinum* toxins are low heat resistance and according to them, these toxins were inactivated at temperature, $50^{\cdot C}$ and above when heated for 5 minutes. The variation in the different heat resistance of toxins found in their studies and that of ours could be explained on the ground that typical toxins might have been different in different cases.

Table.3-Toxicity of *Clostridium botulinum* inoculates in mice ($45x10^6$ per ml).

S. No.	Weight of mice	Method of injection	Amount injected	Symptoms observed
1.	63.5 gm	Intraparetoneal	2.0 cc	Nausa, diarrhoea, and vomiting observed after 3.5 hours death after 18 hours.
2.	62.7 gm	Intraparetoneal	1.5 cc	Vomiting and diarrhoea after 4.5 hours and death after 20 hours.
3.	63.7 gm	Intraparetoneal	1.0 cc	No effect.
4.	29.5 gm	Intraparetoneal	2.0 cc	Slight effect of diarrhoea and vomiting, paralysis of leg after 8 hours. Recovered within 24 hours
5.	30.4 gm	Intraparetoneal	1.0 cc	No effect

Table.4-Toxicity of *Staphylococcus aurius* inoculates in mice ($69 x10^6$ per ml).

S. No.	Weight of mice	Method of injection	Amount injected	Symptoms observed
1.	64.4 gm	Intraparetoneal	2.0 cc	Vomiting, nausa with slight diarrhea and prostration observed after8 hours, death after 20hours.
2.	62.9 gm	Intraparetoneal	1.5 cc	Vomiting and diarrhoea after 6.5.5 hours and death after 24 hours.
3.	63.1 gm	Intraparetoneal	1.0 cc	No effect.
4.	29.1 gm	Intraparetoneal	2.0 cc	Very slight effect as vomiting and prostration after 10 hours. Recovered within 24 hours.
5.	30.2 gm	Intraparetoneal	1.0 cc	No effect

IV. CONCLUSION

It can be concluded from the experimental results that the bacteriological quality in terms of Clostridium botulinum and Staphylococcus aurious supplied to Tura town is very poor. Although the microbial quality of milk products was not up to the mark but these are safer from health hazards as their Clostridial and Staphylococcal counts were noted quite low to produce any health hazards as more than 10^5 Clostridium botulinum and $5x\ 10^7$ Staphylococcus aurious per ml of milk are required to produce detectable levels of toxins. The botulinal toxin can be destroyed by heating where as it is difficult to destroy Staphylococcal enterotoxins by heat treatment. It is rather better to avoid contamination and destruction of bacteria than inactivation of toxin.

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