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Isolation of Bacteria from Milk Based Indian Sweets Sold in and Around Kolkata Using MPN Method

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Abstract: Milk is frequently used as a vital ingredient in the preparation of Indian sweets, which are renowned for their rich flavour and cultural significance. To guarantee consumer health, it is essential that these traditional treats are microbiologically safe. This study used the multiple tube test to evaluate the microbiological quality of milk-based Indian sweets sold in and around Kolkata. 80 samples in all were gathered, and the microbial contamination was examined. According to the findings, 64 of the 80 samples (or 80%) tested positively for different bacterial species. The identified bacterial strains included Acinetobacter baumannii (2.5%), Aeromonas schubertii (1.25%), Citrobacter freundii (2.5%), Citrobacter koseri (8.75%), Enterobacter cloacae (15%), Escherichia coli (12.5%), Klebsiella pneumoniae (2.5%), Klebsiella aerogenes (28.75%), and Klebsiella oxytoca (6.25%). The quality and safety of the region's milk-based Indian sweets are questioned by the presence of these pathogenic and opportunistic bacteria. The likelihood of foodborne infections linked to these sweets is shown by the prevalence of Enterobacteriaceae members. Since these traditional treats are prepared, stored, and distributed by regulatory agencies and sweet vendors, strict hygiene and quality control standards must be put in place.

Keywords: Sweets, Food Safety, Public Health, Quality, Vendors.

1. INTRODUCTION

Milk-based Indian sweets hold a special place in the hearts and palates of people across the Indian subcontinent, and they are an integral part of various cultural and festive celebrations. These delectable delicacies are prepared using milk as a primary ingredient, making them not

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only delicious but also a potential vehicle for microbial contamination(1). Ensuring the microbiological safety of these traditional desserts is of paramount importance to safeguard public health and maintain the integrity of the food industry.

In recent times, there has been growing concern about the safety and quality of food products, including Indian sweets. The risk of foodborne illnesses caused by pathogenic microorganisms has prompted a need for comprehensive assessments of the microbiological quality of these food items(2). Kolkata, with its rich culinary heritage and vibrant street food culture, serves as an ideal location to investigate the microbial contamination of milk-based Indian sweets sold in and around the city.

The objective of this study was to conduct a thorough assessment of the microbiological quality of milk-based Indian sweets available in Kolkata using the multiple tube test. The multiple tube test, also known as the Most Probable Number (MPN) method, is a widely accepted and reliable technique for enumerating coliform bacteria in food samples. By employing this method, we aimed to identify and quantify the presence of various bacterial species, including potential pathogenic organisms, to ascertain the overall safety of these beloved confections (3).

Food safety is a crucial aspect of public health, and the findings from this research are expected to shed light on the level of microbial contamination in milk-based Indian sweets and raise awareness among consumers, vendors, and regulatory authorities. Moreover, the identification of specific bacterial strains will help in understanding the sources and contributing factors of contamination, facilitating the development of targeted intervention strategies to mitigate risks and enhance the overall quality of these cherished desserts(4).

In the subsequent sections, we will detail the methods used for sample collection and analysis, present the results obtained from the multiple tube test, and discuss the implications of our findings for the consumers, food industry, and public health authorities. Additionally, we will propose recommendations and potential measures to ensure the microbiological safety of milk-based Indian sweets, thereby safeguarding the health and well-being of the consumers and preserving the cultural significance of these delectable treats.

2. MATERIALS AND METHODS

Type of Study: Public health and microbiological study.
Study Design: Laboratory Based observational study.
Place of Study: Kolkata.
Samples: Milk based sweets samples
Method of Sampling: Random Sampling.
Sample Size: Sample size is 80.

3. RELATED WORK

Collection of Samples

All the aseptic precautions were taken to collect the sample avoiding external contamination. Sample were collected in sweets carry box. Each of the sample were directly brought to

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microbiology lab and then transferred to sterilized sample collection vials. Sample collection vials were labelled indicating the serial number of samples. At least 2-4 different nos. of sample were collected from different shops and vendor on the basis of variety of Sandesh they had in their menu. Maximum of 4 samples were collected from a particular shop.

Preparation of Samples
For first 14 samples (M 01 – M 14) the complete sweets (weight of sweets ranged from 18-22 g per piece) were diluted in 125 ml of normal saline (NS). Rest sweets sample (M 15 – M 80) were weight to 1.2 g to 1.3 g and diluted in 125 ml of normal saline.

Inoculation on Culture Media
One hundred five (105) ml of diluted sample was cultured for coliform count by multiple tube tests. 50 ml diluted sample is added to one bottle containing a 50 ml double strength MacConkey broth. 10 ml diluted sample added to 5 bottles containing 10 ml double strength medium MacConkey broth. 1 ml diluted sample added to 5 bottles containing 5 ml single strength MacConkey broth. The total volume tested was now 105 ml of diluted sample. The total number of tubes used for diluted sample culture was 11. After inoculation of sample, they were incubated at 37º C for 48 hours.

4. RESULTS

Result Interpretation of Multiple Tube Method for coliform count (5)
By referring to the McCrady's Probability Table the MPN can be derived and the following results were noted down.
Excellent: If MPN zero (no growth in all the tubes).
Satisfactory: If MPN is 1-5 (number of tubes showing growth).
Indeterminate: If MPN is 6-10 (number of tubes showing growth) and needs more treatment to purity the sources.
Unsatisfactory: If MPN is above 10, this needs vigorous treatment to clear the contamination.
Identification of isolates
All of the distinguishing isolates were subjected to additional investigation for identification. The isolates were recognized based on physical characteristics, cultural traits, and biochemical responses.

Table 1.1: - Different Range of Colony Forming Unit

Coliform Count (MPN) by multiple tube test		
S. No	**Range**	**No. of Sample**
1	0	16
2	1-9	11
3	10 – 99	19
4	100 – 180	9
5	180+	25
Totals		80

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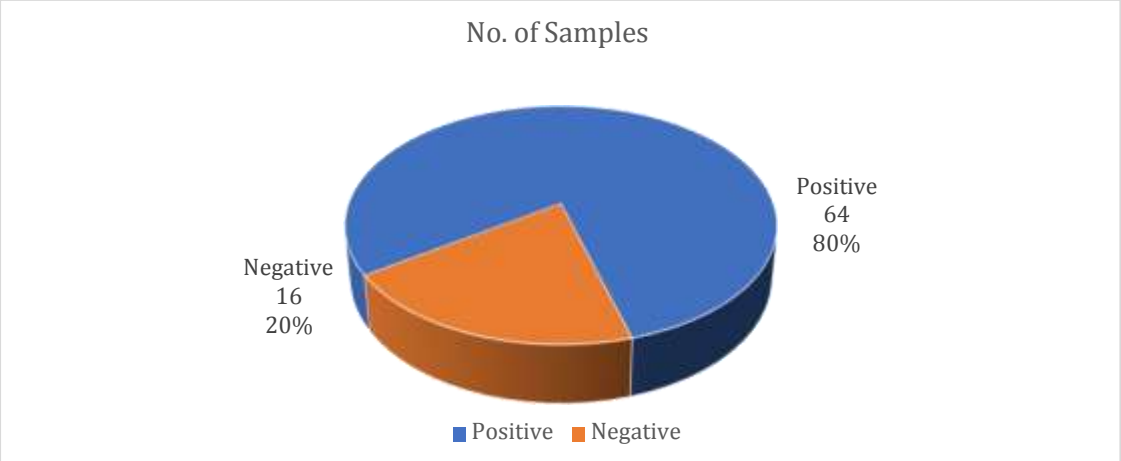

Fig 1.1: - Pie chart showing no. of positive & negative samples for Coliform bacilli by MPN Method

he above chart shows that 80 samples which were cultured on milk agar shows 80% positivity which means that out of 80 sample 64 samples were found positive and rest 16 samples were negative which means that there was no bacterial growth on media for these 16 samples.

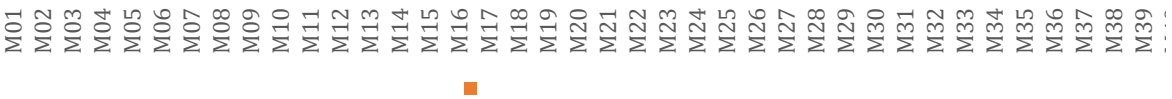

Fig 1.2: - Bar Diagram of No. of Coliform bacilli Count for M01 – M40

The above chart represents probable no. of coliform bacilli in 100 ml of diluted sample from M01 to M40 on multiple tube test method, the highest no of coliform bacilli was 180+ and lowest was zero (0).

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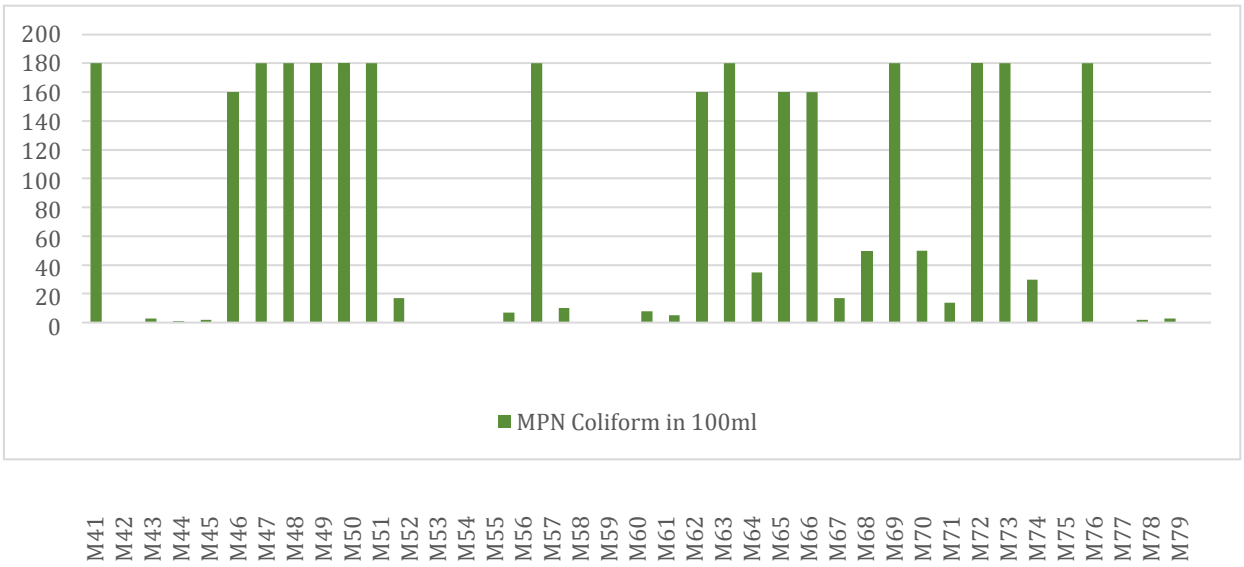

Fig 1.3: - Bar Diagram of No. of Coliform Count for M41 – M80

The above chart represents probable no. of coliform bacilli in 100 ml of diluted sample from M41 to M80 on multiple tube test method, the highest no of coliform bacilli was 180+ and lowest was zero (0).

Table 1.2: - Total No. of Coliform bacilli & Others Isolates & Their Percentage

S. No	Name of Bacteria	No. Of isolates Present	Percentage of isolation out of total number of samples
1	Acinetobacter baumanni	2	2.5 %
2	Aeromonas schubertii	1	1.25 %
3	Citrobacter freundi	2	2.5 %
4	Citrobacter koseri	7	8.75 %
5	Enterobacter cloacae	12	15 %
6	Escherichia coli	10	12.5 %
7	Klebsiella pneumoniae	2	2.5 %
8	Klebsiella aerogenes	23	28.75 %
9	Klebsiella oxytoca	5	6.25 %
10	Negative	16	20 %
Total		80	100 %

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Out of 80 samples 64 samples were positive. In those Acinetobacter baumanni (2.5%), Aeromonas schubertii (1.25%), Citrobacter freundi (2.5%), Citrobacter koseri (8.75%), Enterobacter cloacae (15%), Escherichia coli (12.5%), Klebsiella pneumoniae (2.5%), Klebsiella aerogenes (28.75%), and Klebsiella oxytoca (6.25%) were present.

5. DISCUSSION

The purpose of the current study was to use the multiple tube test to evaluate the microbiological quality of Indian sweets made with milk that were sold in and around Kolkata. Our investigation's findings showed a significant amount of microbial contamination in these classic treats, suggesting possible dangers to food safety connected with eating them.
Alarmingly, 64 (80%) of the 80 samples that were examined revealed the presence of germs. A wide variety of bacterial species are present, which emphasizes the need for increased awareness in monitoring the hygiene and production practices used during the manufacture and preservation of these delectable delicacies.
The two most common bacterial species found in the samples were Klebsiella aerogenes and Enterobacter cloacae, which together made up a significant 28.75% of the positive samples. Both Klebsiella aerogenes and Enterobacter cloacae are known opportunistic infections linked to foodborne diseases and are members of the Enterobacteriaceae family(6). Concerns regarding the general microbiological safety of these milk-based Indian sweets are greatly heightened by their presence in such a high percentage of the samples.
12.5% of the positive samples contained Escherichia coli, another member of the Enterobacteriaceae family. The presence of E. coli is particularly concerning since some strains of this bacterium, especially in vulnerable populations, can result in severe food poisoning and serious health issues(7).
Other potentially dangerous bacteria were found in the samples in addition to members of the Enterobacteriaceae family(8). In 2.5% of the positive samples, the multidrug-resistant bacterium Acinetobacter baumannii was found(9). In 1.25% of the samples, Aeromonas schubertii was discovered, which is frequently discovered in aquatic habitats(10,11). Klebsiella pneumoniae, Klebsiella oxytoca, Citrobacter freundii, and Citrobacter koseri were found in 2.5%, 8.75%, 2.5%, and 6.25% of the positive samples, respectively. The presence of any pathogenic microorganisms in food items poses a possible health concern, even though the prevalence of these bacteria may appear to be minimal.
There are a number of reasons why milk-based Indian sweets might become contaminated with pathogenic and opportunistic bacteria, including poor handling techniques during preparation, poor storage conditions, and cross-contamination from machinery and surfaces in the production area(12).
Strict food safety procedures must be put in place across the whole production and distribution chain in order to address the problem of microbial contamination in Indian sweets made with milk. This involves maintaining ideal storage temperatures, adhering to good hygiene and sanitation procedures during the preparation process, and routinely checking the manufacturing environment for potential sources of contamination(13,14)
To reduce the danger of bacterial contamination, food manufacturers and vendors must get

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education and training on the best practices for food safety. Furthermore, regulatory agencies should create and enforce for these items' food safety requirements, doing routine inspections to verify compliance(15).

The samples' high concentration of these bacteria highlights the requirement for meticulous attention to food safety procedures during the preparation, storage, and distribution of Indian sweets made with milk. To reduce the danger of microbial contamination and ensuing foodborne illnesses, proper handling, storage, and hygiene practices should be put in place(17).

It is essential to inform and train food manufacturers and vendors on best practices for food safety in order to guarantee the microbiological safety of these cherished delicacies. In order to ensure compliance, regulatory bodies should create and enforce strict criteria for food safety.

6. CONCLUSION

The results of this study highlight the pressing need to address the microbiological quality of Indian sweets made with milk that are sold in and around Kolkata. The risk to one's health from consuming these common desserts is highlighted by the high frequency of pathogenic and opportunistic germs(16). We may work to ensure the safe consumption of these customary foods while retaining their cultural value by establishing efficient food safety procedures, increasing awareness among customers and food vendors, and enforcing regulatory standards. To follow the success of these treatments and ensure the regional supply of milk-based Indian sweets is microbiologically safe, ongoing research and observation are required. Protecting consumer health and promoting food safety should be the collective responsibility of all stakeholders involved in the production and sale of these beloved delicacies. All parties engaged in the manufacture and distribution of Indian sweets made with milk should take action in light of the study's findings. It is possible to reduce the hazards associated with microbiological contamination and improve the general quality and safety of these traditional sweets by implementing efficient food safety procedures, increasing consumer knowledge, and enforcing regulations.

The microbiological quality of Indian sweets made with milk must continue to improve, thus more investigation and monitoring are required to assess the efficacy of initiatives. By placing a high priority on food safety, we can enjoy these delectable delicacies without endangering consumer health and keep their cultural relevance for upcoming generations. Milk based sweets contains coliforms which can come from milk, milk handler or sweets processing plant.

Abbreviations

AIIH & PH: All India Institute of Hygiene and Public Health
E. coli: Escherichia coli

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NS: Normal Saline
MPN: Most Probable Method
M 01, M 02…..: Name of sample

7. REFERENCES

1. Pal M, Mulu S, Tekle M, Pinto S, Prajapati J. Bacterial Contamination of Dairy Products. Beverage Food World. 2016 Jan 1;43:40–3.
2. Microbial testing – An overview of what it is and what it is for [Internet]. [cited 2023 Jul 19]. Available from: https://www.biosafe.fi/insight/microbial-testing
3. Erkmen O. Practice 4 - Most probable number technique. In: Erkmen O, editor. Microbiological Analysis of Foods and Food Processing Environments [Internet]. Academic Press; 2022 [cited 2023 Jul 19]. p. 31–7. Available from: https://www.sciencedirect.com/science/article/pii/B9780323916516000422
4. Food safety [Internet]. [cited 2023 Jul 19]. Available from: https://www.who.int/news-room/fact-sheets/detail/food-safety
5. Water Bacteriology: Introduction, Testing Methods, Test Requirements, Test [Internet]. [cited 2023 Jun 17]. Available from: https://universe84a.com/water-bacteriology-introduction/
6. Nyenje ME, Odjadjare CE, Tanih NF, Green E, Ndip RN. Foodborne Pathogens Recovered from Ready-to-Eat Foods from Roadside Cafeterias and Retail Outlets in Alice, Eastern Cape Province, South Africa: Public Health Implications. Int J Environ Res Public Health. 2012 Aug;9(8):2608–19.
7. E. coli [Internet]. [cited 2023 Jul 19]. Available from: https://www.who.int/news-room/fact-sheets/detail/e-coli
8. Pennington H. Enterobacteria and bacterial food poisoning. In: Conlon CP, Firth J, Conlon C, Cox T, editors. Oxford Textbook of Medicine [Internet]. Oxford University Press; 2020 [cited 2023 Jul 19]. p. 0. Available from: https://doi.org/10.1093/med/9780198746690.003.0111
9. Elbehiry A, Marzouk E, Moussa IM, Dawoud TM, Mubarak AS, Al-Sarar D, et al. Acinetobacter baumannii as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic of biofilm formation and phenotypic pattern of antimicrobial resistance. Saudi J Biol Sci. 2021 Jan;28(1):1158–66.
10. Martins LM, Marquez RF, Yano T. Incidence of toxic Aeromonas isolated from food and human infection. FEMS Immunol Med Microbiol. 2002 Feb 1;32(3):237–42.
11. Tomas JM. The Main Aeromonas Pathogenic Factors. ISRN Microbiol. 2012 Sep 4;2012:256261.
12. Oliver SP, Jayarao BM, Almeida RA. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog Dis. 2005;2(2):115–29.
13. Jadranka B, Naletina D, Iva N. Food safety and food quality in the supply chain,. In 2015.

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14. Burke N, Zacharski KA, Southern M, Hogan P, Ryan MP, Adley CC, et al. The Dairy Industry: Process,
15. Monitoring, Standards, and Quality. In: Descriptive Food Science [Internet]. IntechOpen; 2018 [cited 2023 Jul 19]. Available from: https://www.intechopen.com/chapters/63169
16. Consumers and food safety: A food industry perspective [Internet]. [cited 2023 Jul 19]. Available from:https://www.fao.org/3/v2890t/v2890t05.htm
17. Kaynar P. Determination of microbiological properties of milk-based desserts presented for consumptionin Ankara, Turkey. 2020 [cited 2023 Jul 19]; Available from: http://earsiv.hitit.edu.tr/xmlui/handle/11491/6929
18. Fischer WJ, Schilter B, Tritscher A, Stadler R. Contaminants of Milk and Dairy Products: Contamination Resulting from Farm and Dairy Practices. Encycl Dairy Sci. 2011 Dec 31;887–97.