Research Paper MICROBIOLOGY

Detection of Adulterants and Microbial Pathogens in Traditional Indian Sweets

Dugal S. Dept.of Microbiology, Sophia College, University of Mumbai, Maharashtra, India.

Shaikh A. Dept.of Microbiology, Sophia College, University of Mumbai, Maharashtra, India.

Worlikar L. Dept.of Microbiology, Sophia College, University of Mumbai, Maharashtra, India.

Patanwala T. Dept.of Microbiology, Sophia College, University of Mumbai, Maharashtra, India.

ABSTRACT

Cases of food intoxication after consumption of traditional Indian sweets have often been reported across India. The present study was undertaken to check the microbial quality of sweets in order to determine whether manufacturers employ proper sanitary procedures in the preparation of these products and the potential risk of acquiring food borne infections on their consumption. Tests were carried out to detect presence of important microbial pathogens S.aureus and E.coli in sweet samples collected from different areas in Mumbai city. 66% of the samples tested positive for coliforms, of which 54% samples showed the presence of E.coli, indicating faecal contamination. Of the total samples tested, 54% were positive for S.aureus, all of which were coagulase positive strains. However, none of the samples showed the presence of any adulterants. The current study underlines the importance for the enforcement of specifications laid down for proper handling and distribution of these sweet products.

KEYWORDS : S.aureus , E.coli , sweets , adulterants , Mumbai.

INTRODUCTION

Varied types of traditional sweets prepared using milk and flour are extensively consumed in India. A wide variation exists in the manufacturing process, microbial quality and the shelf life of these products. In order to cater to heavy demand during festival seasons, sweets are often produced in bulk and under unhygienic conditions. Due to their high nutritive value and improper handling these sweets are prone to contamination with pathogenic bacteria which can cause incidence of severe food poisoning. (Vaidya *et.al*, 2015). Recent researchers have reported the prevalence of *E.coli* and *S.aureus* in traditional Indian sweets sold across the country. (Chauhan *et al*., 2015).

In additional to microbial contamination, adulteration of sweet meats with color, starch or blotting paper is common and can pose a serious health hazard (Neetu et al .2012). Metanil yellow, a common adulterant added to sweets in order to lend them a bright color, is known to damage the internal lining of organs and cause symptoms of giddiness, weakness and food poisoning.

Pathogens like *S. aureus* cause food intoxication due to the production of an enterotoxin which is absorbed by the gut and causes the emetic center of the brain to produce sensation of nausea and vomiting within 6 hours of consumption of food (Ananthanarayan 2006). *Staphylococcal* food poisoning also includes symptoms of abdominal cramps and diarrhea (Bolovan et.al 2000). Presence of pathogens like *S.aureus* is often attributed to animals suffering from disease or to improper pasteurization of milk. Improper refrigeration of sweets is also one of the reasons for enterotoxin production by *S. aureus*.

Presence of *E. coli* in sweets meats represents a potential risk for public health as well as serves as an indication of the presence of other entero pathogens. Detection of *E. coli* is known to be a reliable indicator of fecal pollution, occurring due to poor quality of raw materials and improper handling (Soomro *et.al,* 2000). Entero pathogenic *E. coli* can cause severe diarrhea and vomiting in young children (Singh and Prakash 2008). The current study was designed considering the negative impact that adulteration and microbial contamination of traditional sweets can pose to public health.

METHOD AND MATERIAL

Sample collection

A total of 33 samples of traditional sweets which included khalakhand, peda and gulab jamun were collected by the random sampling method from different locations across Mumbai.

Isolation and identification of microbial pathogen

Media used for isolating organisms and biochemical tests were procured from Himedia (India). Homogenized sweet samples were isolated on salt mannitol agar and MacConkey's agar in order to detect *S.aureus* and *E.coli* respectively. Plates were incubated at 37°C for 24 hours. *S.aureus* was presumptively identified by Gram staining. Cultures identified as *S.aureus* were further subjected to catalase test and free tube coagulase test (Loeb, 1903).

Presence of *E.coli* was confirmed by indole test, methyl red test, Voges-Proskaur test, citrate utilization and by further inoculation on Triple Sugar Iron (TSI) slants (Salle 1984).

Detection of Adulterants

Few drops of concentrated hydrochloric acid was added to a homogenenized suspension of the sweets. Appearance of magenta red color denoted the presence of the adulterant metanil yellow.

Adulteration of kalakhand with blotting paper was detected by adding 3ml hydrochloric acid and 3ml distilled water to a homogenenized suspension of sweets. The contents were stirred with a glass rod which was then examined. Presence of fibers on the glass rod indicated that the sweet was adulterated with blotting paper.

RESULTS AND DISCUSSION

Out of the 33 samples screened, 22 (66%) tested positive for coliforms, which gave pink colored colonies on MacConkey's agar. Out of 22 samples, 12 samples (54%) were contaminated with *E. coli,* while 10 (45%) showed the presence of non- fecal organisms. *E. coli,* a fecal coliform gave a positive indole and methyl red test while Voges-Proskaur test and citrate utilization test were negative. TSI slants, showed yellow butts and yellow slants with gas formation and absence of hydrogen sulphide gas production, thus indicating presence of *E.coli*.

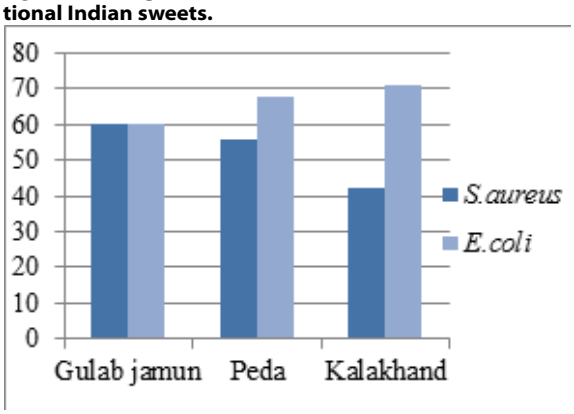
The highest percentage of *E. coli* was isolated from peda (68%). Previously (Neetu *et.al* 2012) have reported presence of *E. coli* in 89.3% samples collected from various vendors in Chandigarh. Poor personal hygiene, use of polluted water and poor quality raw materials usually act as a source of contamination (Chukuezi, 2010).

Out of the 33 samples collected, 18 samples (54%) were positive for *S. aureus*. These isolates showed yellow colored colonies on salt mannitol agar and all 18 of these samples (100%) showed production of enzyme coagulase. Previously 80% of the sweets samples screened have been reported positive by Subbaiah *et.al* 2012 in Bangalore while Chauhan *et.al* 2015 have reported 37.5% incidence of *S. aureus* in sweet samples tested in Goa. The high incidence of *S. aureus* in such foods denotes improper product handling by sweet shops and exposure to unsanitary conditions.

Coagulase test is used to identify and differentiate *S. aureus* from coagulase negative *Staphylococci*. Most strains of *S.aureus* produce 2 types of coagulase, free coagulase and bound coagulase. Free coagulase is an enzyme that is secreted extracellularly. It is detected by the tube coagulase test. Coagulase is an important virulence factor which reacts with coagulase reacting factor in the plasma to form thrombin (Ryan KJ et.al.2004). Thrombin converts fibrinogen to fibrin resulting in clotting of plasma. Detection of coagulase indicates that a particular strain of *Staphylocoocci* is pathogenic and capable of causing disease. Production of coagulase is also significant as previous researchers have reported antibiotic resistance among coagulase positive strains (Mohanta *et.al* 2015).

High bacterial counts in sweets results due to contaminating organisms in fresh produce and continues till the product is handed over to the consumer (Frazier WC, Westhoff DC 1998). Milk is a good substrate for *S. aureus* contamination and toxin production. These bacteria retain their biological activity even after pasteurization (Asao *et.al* 2003). Detection of *E. coli* and *S. aureus* in different traditional Indian sweets is depicted in Figure1.

Fig1: Percentage of *E. coli* and *S. aureus* in different traditional Indian sweets.



There have been concerns expressed regarding adulteration of sweet meats sold in Mumbai city. However, in our study none of the samples, including the ones sold by small-scale manufacturers showed the presence of adulterants like metanil yellow and blotting paper.

CONCLUSION

The findings of our study reveal *E. coli* and *S. aureus* to be frequently occurring pathogens in traditional Indian sweets sold in Mumbai. As observed during sample collection, the handling of sweets with bare hands, non-usage of aprons, absence of hair covering and handing of money during serving, all contribute to poor hygienic conditions. *Staphylococci* could have also entered from food handlers who may have had acute infections or from healthy carriers who harbored these organisms in their nose or throats. Considering public health importance, traditional sweets products need to be prepared hygienically in order to reduce the microbial load. The finding of this study suggests the need for strict adherence to specifications while preparing and handling traditional Indian sweets. This would help in achieving and maintaining uniform product quality and ensuring consumer safety in India.

Much attention is still needed to be applied for attaining desired safety margins and giving assurance that traditional sweets bought by consumers are pure, healthy and of the quality claimed.

REFERENCES

1. Ananthanarayan and Paniker. Textbook of Microbiology, (2006). Orient Longman Pvt. Ltd.,7:192-201.
2. Asao, T, Kumeda, Y., Kawai, T., Shibata, T,, Oda, H., Haruki, K,, Nakazawa, H., Kozaki, S. (2003) An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: Estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiology and Infection* .**13:**33–40.
3. Bolovan, Johanson J.E., (2000). Studies on the pathogenesis of Staphylococcal infection. *J. Exp. Med.* **113**:249-259.
4. Chauhan, D., Manjula, Channapa, N.G., Shivnnavar,Gaddad, S.M.(2015).*Journal of biotechnology and biosafety., **3**: 275-281.*
5. Chukuezi C.O. (2010). Food Safety Hygienic practice of street food vendors in Owerri, Nigeria. *Studies in sociology of science.***1**:50-57.
6. Frazier, W.C., Westhoff, D.C. (1998). Contamination, preservation and spoilage of vegetables and fruits.*Food microbiology,Singapore*, Mcgraw –hill.**1:***196-217*.
7. Loeb L. The influence of certain bacteria on the coagulation of the blood. *The Journal of Medical Research"* 1903; **10(3):**407-419.
8. Mohanta, A.,Majumdar,P.B.,(2015), Detection of staphylococci in raw milk and milk products and evaluation of their antibiotic sensitivity: a report from Southern Assam, India. *Journal of Environmental Science*, *Toxicology and Food Technology.* 9: 17-22.
9. Neetu. Kaul M.,Madhu.(2012).A microbiology investigation on milk based sweets with special Reference to Escherichia coli International Journal of Food And Nutritional Sciences.**1:**146-153.
10. Ryan,K.J., Ray, C.G. (2004). *Sheris Medical Microbiology* (4thed).McGraw hill.***1**:8385-8529*
11. Salle, A.J. *Fundamental Principles of Bacteriology.* Tata McGraw-Hill, 1984.
12. Singh and Prakash 2005.Microbial food safety–Indian Regulations ISO22000:2005. **27:** 7,8,12.
13. Soomro, A.H., Arain, M.A,. Khaskheli, M,, Bhutto, B. (2002) Isolation of Escherichia Coli from Raw Milk and Milk Products in Relation to Public Health Sold under Market Conditions at Tandojam. *Pak J Nutr* **1**: 151-152.
14. Subbaiah,U.M. ,Subramanyam, *V.*Comparison of antibiotic resistance pattern coagulase positive and coagulase negative *S.aureus* from Indian sweets . *Int.J. Res.Pharm. Sc.* (2012). **2(4):**38-47
15. Vaidya, D. N. Ghugarg, P.S. and Kutty, M. (2015). Prevalence of pathogenic microorganism in khoa based meethai sold in Pune city*Journal of global bioscience. **4:***2893-2900.