ABSTRACT

*Staphylococcus aureus* is one of the most common agents in bacterial food poisoning outbreaks. Globally, staphylococcal food intoxication is estimated to cause 185,000 cases of food borne illness annually. Milk is a good substrate for *S. aureus* growth and enterotoxin production. Several *staphylococcal* species other than *S. aureus* reportedly produce staphylococcal enterotoxins (SE). Reports have indicated that both *aureus* and non-*aureus* *Staphylococci* elicit enterotoxins in foods, which are highly thermostable, capable of surviving in foods subjected to thermal treatment, thus leading to food poisoning. Milk and milk-products are well known vehicles of Staphylococcal food poisoning; milk, cheese, burfi, khoa and others being commonly implicated in outbreaks. In our studies, twenty *Staphylococcal* strains were isolated from food samples, among them *S.aureus* and *S.intermedius* were predominant. Expression of *entB* gene from *S. aureus* and *S. intermedius* was carried out using *E.coli* BL21 and pET 101 Topo Expression vector. The expressed Ent B toxin was confirmed by HPLC, SDS – PAGE, Western blot and ELISA. *S.intermedius* did not produce Enterotoxin B either in BHI broth or food samples. Transposon like element was thought to suppress the expression of enterotoxin in non- *S. aureus* strains. The *S.intermedius entB* sequence showed similarity with reported *S. aureus entB* gene. *S.intermedius* food isolates showed presence of other *entA* and *entC* genes. Sequence showed similarity with reported *S. aureus entA* and *entC* genes. Plasmid encoded enterotoxigenic characters were studied in *S.intermedius*, a non-*S.aureus* isolates. Plasmid encoded characters like toxin genes and transposon like element were also present in non *S. aureus* food isolates. Among different antibiotics studied for the presence plasmid encoded resistance, the isolates showed resistance to nalidixic acid, tetracycline and kanamycin. The isolates also showed resistance to metal ions such as cobalt, zinc, silver and potassium.

The importance of food safety in the present global scenario has placed emphasis on the need for rapid and specific detection methods for food borne pathogenic bacteria. Isothermal amplification (LAMP) of toxigenic genes was attempted to fasten the detection process. In order to determine the optimal conditions of LAMP, genomic DNA of *S. aureus* MTCC 96 was used as the target template.
Standardization and optimization of LAMP assay for entA, entB, entC and entD genes was carried out using LAMP primers. The reaction mixture was incubated at temperatures ranging from 50 to 60°C. The maximum amplification was found to be 60°C. LAMP reaction was carried out at various incubation periods, i.e., 15, 30, 45 and 60 min at 60°C. Good quantity of amplified product was observed and the product reached maximal at 60 min. Therefore, optimized LAMP conditions were maintained at 60°C for 60 min in further studies. The detection limit was 100 colony-forming units (cfu)/test for LAMP and 10,000 cfu/test for PCR. In the presence of coexisting microbe such as Yersinia enterocolitica did not interfere in the assay and the sensitivity of LAMP remained the same. Enterotoxigenic staphylococci were isolated from food samples. The results were strongly correlated with PCR and LAMP reaction. LAMP positive reaction mixture (amplification product detected by agarose gel electrophoresis) showed the formation of green colour upon the addition of SYBR Green I, while LAMP negative reaction mixture remained orange. Simple, rapid and cost effective direct DNA isolation method was developed by spiking different food samples using standard S. aureus strain. The direct DNA isolation method was standardized by using different concentrations of Triton X-100 (0.01, 0.1, 1, 2, 5 and 10%) and different time intervals (5, 10, 15, 30 and 45 min). Among these 1 % Triton X-100 at the time of 10 min lysed the cells effectively and gave expected DNA. To extract bacterial DNA from the spiked food samples, the centrifuged sample pellets were boiled with required volume of 1% Triton X-100 for 10 min. The lysate were used for PCR and LAMP reactions. PCR and LAMP assays with entA, entB, entC and entD primers in conjunction with DNA extracts from food samples such as milk, khoa, milk burfi, rice and sugarcane juice having known concentrations of S. aureus were carried out. The detection limit of PCR was $10^4$ cfu/g, $10^4$ cfu/ml and LAMP was $10^2$ cfu/g, $10^2$ cfu/ml. Despite the presence of other bacteria, the sensitivity of PCR and LAMP to S. aureus remained the same. In the present study, twenty five food samples collected from different sources from the local market were analysed. In food sample where S. aureus count was $10^3$ cfu/g or less, only LAMP reaction showed positive amplification and PCR failed to detect at this concentration of cells.