

Detection of *Staphylococcus aureus* Enterotoxins (SE_s) in Foodstuffs by ELISA

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Staphylococcal enterotoxigenesis results from ingestion of foods containing 1 of 11 immunologically distinct enterotoxins, A, B, C₁, C₂, C₃, D, E, F, G, H and I. *Staphylococcus aureus* is heat-labile and its enterotoxins are heat-stable^{1,2,3}, due to presumption enterotoxins culture results for viable bacteria are not sufficient to prove food safety for consumption. In this study we examined the presence of *Staphylococcus aureus* and staphylococcal enterotoxins (SEs) in 60 samples of ready to use foodstuffs that should be free of this organism, obtained from samples were suspected to have foodborne pathogens and referred to FDCLs of Iran. Viable bacteria were detected by culture, and simultaneously enterotoxins by ELISA, using commercial kit for the detection of A, B, C, D, E, F and G enterotoxins, after extraction and purification from the food substrate. *Staphylococcus aureus* were detected by enrichment method¹⁰. Giolitti cantoni broth was used as enrichment media and Baird Parker agar as selective media. 27 samples were positive in routine culture method and in 38 samples enterotoxins were found by ELISA method. All enterotoxin-positive samples had enterotoxin A and 21 of them enterotoxin B too. Based on the results, the culture method for detection of food contamination is not a definite reliable method, and ELISA method could be preferred, in this respect.

Key words: *Staphylococcus aureus*, Enterotoxins (SE_s), Foodstuff, ELISA.

Staphylococci receive relatively little attention in comparison with other organisms, such as *Salmonella*, *Campylobacter* and vero cytotoxigenic *Escherichia coli*, but their importance should not be underestimated at all.

Under-reporting of foodborne disease it is recognized as a significant problem in all countries, especially where symptoms are mild and recovery is rapid. The importance of *Staphylococcus aureus*, has been reduced by improved control through refrigeration, but the organism remains a significant cause of foodborne morbidity^{1,2,3,8}.

Staphylococci are predominantly of animal origin, although isolation of some species may be made from environmental sources. Staphylococcal enterotoxigenesis (Staphylococcal

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food poisoning) results from ingestion of enterotoxins, synthesised during growth in foods. Staphylococcal enterotoxigenesis results from ingestion of foods containing 1 of 11 immunologically distinct enterotoxins, A, B, C₁, C₂, C₃, D, E, F, G, H and I. Most outbreaks involve SEA and SED, which are produced over a wider range of conditions^{1,2,3,8}.

METHOD

60 samples of ready to use foodstuffs obtained from samples were suspected to have foodborne pathogens and referred to FDCLs of Iran were examined for *Staphylococcus aureus* and staphylococcal enterotoxins (SEs) presence. Viable bacteria were detected by culture, and simultaneously enterotoxins by ELISA using commercial kit, Rida screen set A, B, C, D, E from r-Biopharm AG, Darmstadt, Germany with 0.2 – 0.7 ng/ml detection limit, for the detection of A, B, C, D, E, F and G enterotoxins, after extraction and purification from the food substrate. *Staphylococcus aureus* were detected by enrichment method. Giolitti cantoni broth was used as enrichment and Baird Parker agar as selective media. Many selecting media exist, of which Baird Parker (egg yolk-glycerine-tellurite-pyruvate) medium is most widely used as an effective media¹⁰.

RESULTS

27 samples were positive in routine culture method and in 38 samples enterotoxins were found by ELISA method. All enterotoxin-positive samples had enterotoxin A and 21 had enterotoxin B too..

DISCUSSION

Staphylococcus aureus is not heat resistant and is destroyed at using temperatures which are applied normally during food processing, including milk pasteurization and in the recommended condition for meats processing. The organism is also destroyed at treatment levels proposed for most other means of processing food for safety, such as irradiation. *Staphylococcus aureus* is relatively resistant to high-pressure

processing. Toxins are stable and are not destroyed by processing of severity usually applied in food processing^{1,2,3}.

CONCLUSION

Although the culture method for the diagnosis of bacteria is the gold standard method, but serological methods for detection of enterotoxins in foods based on enzyme-linked assay or even latex agglutination due to their high sensitivity, is recommended. Application of these methods increase the effectiveness of detection while reduce the time of analysis.

REFERENCES

1. Adams, M.R., Moss, M.O., Food Microbiology, second edition, Royal Society of Chemistry 2000.
2. Blackburn, C.W., McClure, P.J., Foodborne Pathogens, Hazard, risk analysis and control, CRC 2002.
3. Miliotis, M.D., Bier, J.W., International Handbook of Foodborne Pathogens. Marcel Dekker Inc., 2003.
4. Forbes, B.A., Sahu, D.F., Weissfeld, A.S., Bailey and Scott's diagnostic microbiology 11th ed. Mosby 2002.
5. Koneman, E.W., Allen, S.D., Janda, W.M., et al., Diagnostic Microbiology. Fifth ed., Lippincott, USA 1997.
6. Murray, R.P., Rosenthal, K.S., Kobayashi, G.S., Pfaller, M.A., Microbiology, Fourth ed. Mosby Inc 2002.
7. Pepe, O., Blaiotta, G., Bucci, F. et al. *Staphylococcus aureus* and *Staphylococcal enterotoxin A* breaded chicken products: detection and behavior during the cooking process. *Applied and Environmental Microbiology*, 2006; **72**(11): 7057-7062.
8. Walker, T. S., Microbiology, Saunders text and review series, 1998.
9. Winn, W., Allen, S., Janda, W., Koneman, E., et al. "Koneman color atlas and textbook of Diagnostic Microbiology." sixth ed., Lippincott Williams & Wilkins, USA 2006.
10. ISO 6888-3: Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of Coagulase-positive Staphylococci (*Staphylococcus aureus* and other special-Part 3: Detection and MPN technique for low numbers 2003.