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RESEARCH ARTICLE

OCCURENCE OF *STAPHYLOCOCCUS AUREUS* AND *SALMONELLA* SPECIES IN RAW BEEF

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ABSTRACT

Staphylococcus aureus and *Salmonella* are major food borne pathogens of great public health concern worldwide. In the study PCR protocol was standardized for the detection of these organisms using oligonucleotide primers targeted against *nuc* gene of *S.aureus* and *inv* A gene of *Salmonella* spp. Specificity of primers was tested using reference strains (MTCC 1144 and MTCC 98) and other bacterial strains including *E.coli*, *E.coli* O157:H7 and *V. parahaemolyticus*. PCR detection on artificially inoculated beef samples showed a sensitivity value up to 1 cfu/g for both organisms. Out of 190 beef samples, 99 (52.10%) tested positive by culture method and 109 (57.36%) by PCR for *S. aureus*. With respect to *Salmonella* spp., rate of occurrence was 29.47% (n=56) and 36.31% (n=66) by culture method and PCR respectively. The high percentage of occurrence of *S. aureus* and *Salmonella* spp. highlights the need to improve the sanitation and hygiene procedures at all levels from production to the consumption of meat.

INTRODUCTION

Food-borne pathogens are the leading cause of disease and mortality in developing countries, causing death of approximately 1.8 million people annually (Iyer *et al*., 2013). Changes in eating habits, mass catering complexes and lengthy food supply procedures with increased international movement and poor hygienic practices are major contributing factors for the occurrence of food poisoning (Hedberg *et al*., 1992). *Staphylococcus aureus* and *Salmonella* spp. are the frequently recognized bacterial food borne pathogens responsible for food poisoning and food related intoxications.

Staphylococcus aureus is a Gram-positive, catalase-positive commensal bacterium colonizing both humans and animals. 'The organism" is known for causing food poisoning through the production of enterotoxins. It is responsible for causing a variety of animal diseases such as mastitis, arthritis and urinary tract infections and a prominent cause of food borne intoxications due to poor hygienic practices (Akbar *et al*., 2013). *Staphylococcus* food poisoning is commonly associated with fresh and ready-to-eat foods particularly meat products. This is considered as the third largest cause of food related illness worldwide (Aydin *et al*., 2011).

Food borne Salmonellosis is recognized as a global zoonosis and constitutes a serious public health threat due to its endemic nature, high morbidity and association with a wide range of foods (Moussa *et al*., 2012). Consumption of raw and under cooked meat and meat products remain the principal source of *salmonella* but it has been also associated with dairy products and vegetables (Robles *et al*., 2008). Improper cooking, inadequate storage, cross-contamination and use of raw ingredients in the preparation of foods are the most common factors contributing to outbreaks.

Monitoring the presence of foodborne pathogens in foods is the primary tool for the implementation of food safety systems. It is necessary to monitor the prevalence of food borne pathogens for effective food safety planning and targeted interventions. Recently, with the development of molecular techniques, polymerase chain reaction (PCR) has become an important tool for detecting pathogenic microorganisms in food products by replacing the time-consuming culture-based classical techniques (Xu *et al*., 2006). They are rapid, easy to handle, sensitive and specific and constitute very valuable tools for microbiological applications. The objective of this study is to investigate the occurrence of *S. aureus* and *Salmonella* spp. in raw beef using PCR and culture techniques and to compare the results.

MATERIALS AND METHODS

Reference strain and preparation of inoculums

Staphylococcus aureus (MTCC 1144) and *Salmonella enterica* Typhimurium (MTCC 98) were procured from Microbial type culture collection and Gene bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. Cultures were grown in Tryptic soy broth with yeast extract (TSBYE) at 37°C for 18h. Then, tenfold serial dilutions were prepared in normal saline solution to obtain dilutions containing 10^0 to 10^4 cfu/ml.

Artificial inoculation of meat samples

Meat samples were purchased from retail market and aseptically transferred to the laboratory in sterilized polythene bags and kept frozen at -20^0 C. The samples were tested microbiologically for the presence of organisms under study. After ensuring the absence of the organisms, twenty five grams of meat was weighed and different concentrations of *S. aureus* and *Salmonella* spp. *i.e.*,1cfu, 10 cfu, 10^2 cfu and 10^3cfu/g were inoculated individually with 225ml of sterile Tryptic Soy Yeast extract broth (TSBYE) and pummeled in a stomacher (AES Chemiuex, France) for two minutes. An uninoculated sample was also included as control to ensure the sterility. Samples were then incubated at 37°C for 18h.

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Microbiological analysis

Microbiological analysis was carried out by the method demonstrated by APHA, 2001. Pre-incubated samples (0.1 ml) in TSBYE were spread on the surface of Baird-Parker agar medium (Himedia, India) supplemented with Egg Yolk Tellurite Emulsion (Himedia), selective media for *S. aureus* and incubated further at 37°C for 48 h. Black colonies surrounded by opaque halo on Baird-Parker agar were considered as presumptive *S. aureus*. For the isolation of *Salmonella* spp., 0.1 ml of enriched samples were spread on the surface of Brilliant green sulpha agar medium (Himedia, India), selective media for *Salmonella* and incubated at 37°C for 24 h. Pink coloured colonies were counted as *Salmonella* spp. and isolated colonies were subjected to series of biochemical tests for confirmation.

Polymerase chain reaction

Extraction of DNA was carried out by boiling and snap chilling method (Lee *et al*., 2009). Target genes that were detected by PCR, primer sequences, amplicon size and the references thereof are summarized in Table.1.

Table 1 Primers used in the study

Organism	Target gene	Sequence	Amplicon size	Reference
S. aureus	*nuc*	ForwardGCGATTGATGGTGATACGGTT Reverse AGCCAAGCCTTGACGAACTAAAGC	270bp	Brakstad *et al*. (1992)
S. enterica Typhimurium	*inv* A	ForwardGGTGGCAAGGGAATGAA ReverseCGCAGCGTAAAGCAACT	284bp	Rahn *et al.* (1992)

PCR amplifications were conducted in a reaction mixture containing 2.0µl PCR buffer (10x, Sigma), 2.0µl $MgCl_2$ (2.5mM, Sigma), 0.5µl dNTPs (2mM each, Fermentas), 0.5µl of each primer sets (10pM, Sigma), 0.2µlTaq Polymerase (5U/µl, Sigma) and 2µl of template DNA, in a final volume of 25µl. Amplification conditions were 2 min. at 95^0C, 35 cycles of 15sec. at 95^0C, 30sec. at 60^0C and 60sec. at 72^0C and a final extension of 10 min. at 72^0C. Reactions were carried out in a Master cycler (Eppendorf, Germany). After PCR reaction, 3µl of the PCR product was resolved on 1.5% agarose gel. The gels were stained with Ethidium bromide (0.2µg/ml) and photographed under UV transillumination in gel documentation system (Synoptics, UK). To evaluate the specificity of primers used in the study, PCR was carried out using the DNA prepared from the common cross-reacting bacterial species such as *E.coli*, *E.coli* O157:H7, and *V. parahaemolyticus*.

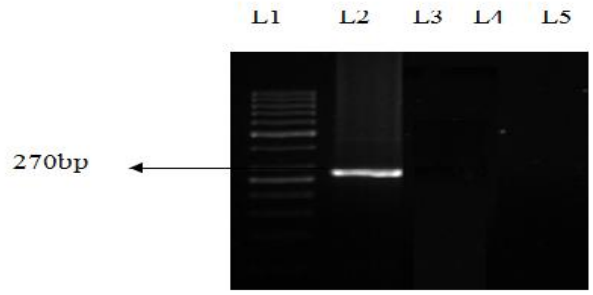

Figure 1 Specificity of PCR using *nuc* primer(L1-Marker, L2-*S. aureus*, L3- *E.coli*, L4- *E.coli* O157:H7and L5- *V. parahaemolyticus*)

Screening of samples

A total of 190 beef samples were collected from retail markets in Mannuthy (n=22), Thrissur (n=63), Calicut (n=20), Ernakulam (n=66) and Wayanad (n=19). Samples were collected aseptically and transported to the laboratory under chilled condition. All samples were investigated for the presence of *S.aureus* and *Salmonella* spp. using in parallel the microbiological method and PCR. Samples were considered positive when the suspected colonies were confirmed by biochemical identification and PCR amplification with specific primers.

Statistical analyses

All data were analysed by Mc Nemar's test using SPSS version 22.0. Evaluation of PCR and culture techniques was done by calculating sensitivity, specificity, efficiency and predictive values.

RESULTS AND DISCUSSION

Despite improvements in meat processing hygiene practices in recent years, the occurrence of pathogenic organisms is a major cause of food borne diseases and highlights the need for a rapid and accurate identification of these food borne pathogens (Guan *et al*., 2013). In the present study, cultural and PCR techniques were employed to investigate the occurrence of *S. aureus* and *Salmonella* spp. in raw beef.

In the recent years, PCR protocol is emerged as a rapid, easy to handle, sensitive and specific technology for detecting food-borne pathogens. Implementation of these methodologies to the routine laboratory analysis still needs more data on their application in naturally contaminated food, as well as, their validation with the currently accepted procedures (Alarcon *et al*., 2005). PCR protocol was standardized using the primers targeting the *nuc* gene of *S. aureus* and *inv* A gene of *Salmonella* spp.

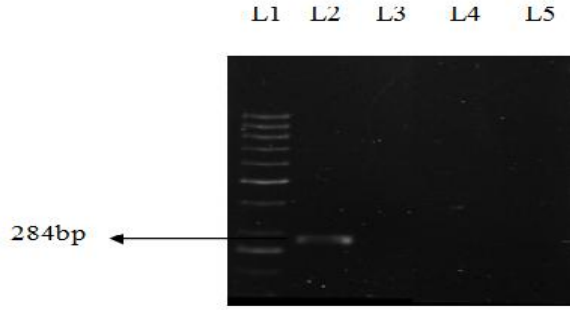

Figure 2 Specificity of PCR using *inv* A primer(L1-Marker, L2-*Salmonella* spp., L3- *E.coli*, L4- *E.coli* O157:H7 and L5- *V. parahaemolyticus*)

Specificity assay of PCR

The standardized PCR protocol for each organism was carried out individually using other competing organisms like *E. coli*, *L. monocytogenes*, and *V. parahaemolyticus* for checking the specificity of *nuc* and *inv* A primers separately. The result showed that the primers were specific for targeting the region of their respective organisms. No nonspecific reactions with other species were obtained in PCR reaction (Figure 1 and 2).

The PCR protocol standardized by Pinto *et al.* (2005) also showed high specificity of *nuc* primers. Santos *et al*. (2001) observed high specificity of *inv* A gene for the *Salmonella* detection.

Sensitivity assay of PCR

When PCR assay was performed using artificially contaminated beef samples after enrichment, upto 1 cfu/g of initial concentration could be detected for both pathogens within 24 h. (Figure 3 and 4). The culture technique was also able to detect same level, but result was obtained after 96 h. of analysis in case of *S. aureus* and 72 h. for *Salmonella*. A study by Alarcon *et al*. (2005) reported that they could detect only 10^3cfu/g of *S. aureus* directly from the homogenized sample without enrichment. Cocolin *et al*. (1998) reported that they could detect *Salmonella* upto 1-10 cfu/25g after 18h. in selenite cysteine broth and 10^2-10^3 cfu/g without enrichment.

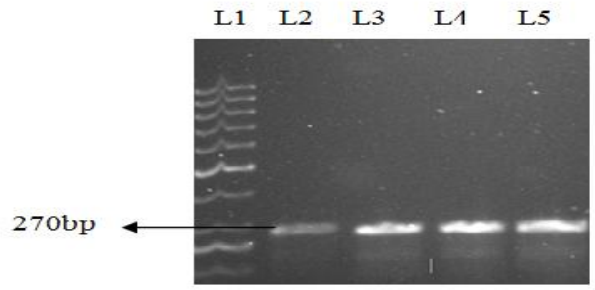

Figure 3 Sensitivity of PCR for *S. aureus*(L1-Marker, L2-1cfu/g, L3-10cfu/g, L4- 10^2cfu/g and L5- 10^3cfu/g)

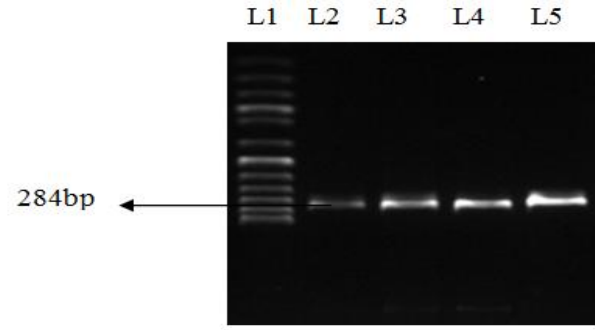

Figure 4 Sensitivity of PCR for *Salmonella* spp.(L1-Marker, L2-1cfu/g, L3- 10cfu/g, L4- 10^2cfu/g and L5- 10^3cfu/g)

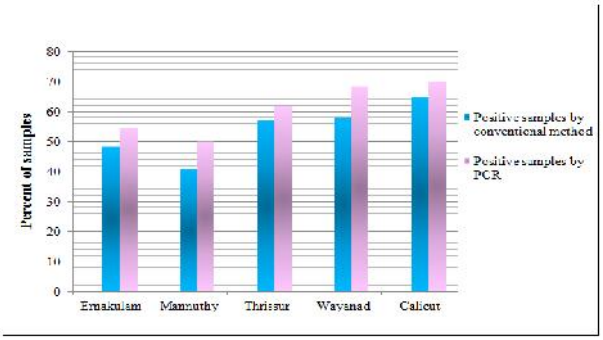

Figure 5 Occurrence of *S. aureus* in beef

Screening of samples

A total of 190 beef samples were analyzed for the presence of *S. aureus* and *Salmonella* spp. by using culture method and PCR. The distribution of the occurrence of both pathogens in meat samples from various sources is presented in Figures 5 and 6.

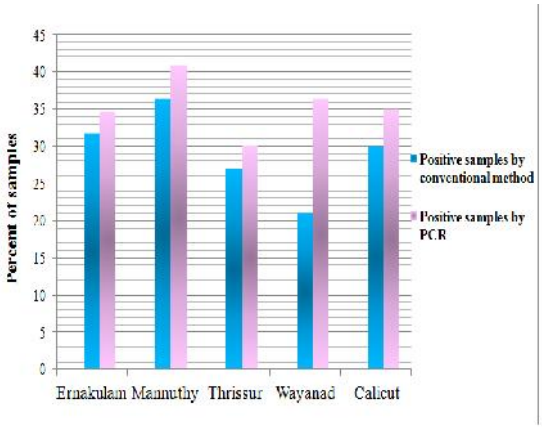

Figure 6 Occurrence of *Salmonella* spp. in beef

Out of 190 beef samples, 99 (52.10%) per cent**.** samples were tested positive by culture method and 109 (57.36%) by PCR for *S. aureus*. Yemisi *et al*. (2011) reported that occurrence of *S. aureus* in meat samples was 46 per cent. A study by Jackson *et al*. (2013) reported 63 % of *S. aureus* contamination of beef samples. A high occurrence rate was reported by Rahimi *et al.* (2013), who found 76.3 % *S. aureus* contamination in beef. With respect to *Salmonella* spp., rate of occurrence was 29.47 per cent (n=56) and 36.31 per cent (n=69) by culture method and PCR respectively. According to Iyer *et al*. (2013), occurrence of *Salmonella* spp. was 45 per cent in beef purchased from butcher shops. Khan et al. (2013) reported a low prevalence rate (4%) of *Salmonella* spp. by PCR and cultural techniques in beef samples.

The comparative study using Mc Nemar's test showed that PCR has sensitivity of 87.88 per cent and 80.36 per cent for *S. aureus* and *Salmonella*, respectively. *Staphylococcus aureus* showed 82.11 % agreement (kappa value = 0.640) between the PCR and cultural methods whereas, *Salmonella* spp. Showed 83.68 per cent agreement (kappa value = 0.625). 93.3 per cent of agreement between PCR and cultural technique was reported for the detection of *S. aureus* by Alarcon *et al*. (2005). Fratamico (2003) reported that there was a good to excellent agreement (kappa value = 0.6824) between both methodologies for the detection of *Salmonella*.

CONCLUSION

In the present study, direct PCR was shown to be very effective in the detection of the pathogens from meat sample homogenates indicating that it is a robust method for rapid detection in comparison with culture technique which provide a significant contribution to both regulatory agencies and the meat industries. The high percentage of occurrence of *S. aureus* highlights the need to improve the sanitation and hygiene procedures at all levels from production to the consumption of meat.

References

Akbar, A. and Anal, A.K. 2013. Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. Asian Pac. J. Trop. Biomed. 3(2): 163–168

Alarcon, B., Vicedo, B. and Aznar, R. 2005. PCR-based procedures for detection and quantification

ofStaphylococcus aureus and their application in food. J. Appl. Microbiol.100(2):352–364

APHA. 2001. Compendium of methods for the Microbiological examination of foods (4 ed.) Washington DC, pp 343-428

Aydin, A., Sudagidan, M. and Muratoglu, K. 2011. Prevalence of Staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne S. *aureus* strains isolated in the Marmara Region of Turkey. Int. J. Food Microbiol. 148:99–106

Brakstad, O.G., Aasbakk, K. and Maeland, J.A. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J. Clin. Microbiol. 30: 1654–1660.

Cocolin, L., Manzano, M., Cantoni, C. and Comi, G. 1998. Use of polymerase chain reaction and restriction enzyme analysis to directly detect and identify *Salmonella* Typhimurium in food. J Appl. Microbiol. 85:673-677

Fratamico, P.M. 2003. Comparison of culture, polymerase chain reaction (PCR), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for detection of *Salmonella* spp. in naturally-contaminated ground chicken, ground turkey, and ground beef. Molec. Cell. Probes. 17 :215–221

Guan, Z.P., Jiang, Y., Gao, F., Zhang, L., Zhou, G.H. and Guan Z.H. 2013. Rapid and simultaneous analysis of five foodborne pathogenic bacteria using multiplex PCR. Eur. Food Res. Technol. 237:627–637

Hedberg, C.W., Levine, W.C., White, K.E., Carlson, R.H., Winsor, D.K., Cameron, D.N., MacDonald, K.L. and Osterholm, M.T. 1992. An international foodborne outbreak of Shigellosis associated with a commercial airline. J. Am. Med. Assoc. 268: 3208-3212

Iyer, A., Kumosani, T., Yaghmoor, S., Barbour, E., Azhar, E. and Harakeh, S. 2013. *Escherichia coli and Salmonella* spp. in meat in Jeddah, Saudi Arabia. J. Infect. Dev. Ctries. 7(11):812-818.

Jackson, C.R., Davis, J.A. and Barrett, J.B. 2013. Prevalence and characterization of Methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. J. Clin. Microbiol. 51(4):1199–1207

Khan, J.A., Rathore, R.S., Khan, S. and Ahmad, I. 2013. Prevalence, characterization and detection of *salmonella* spp. from various meat sources. Adv. Anim. Vet. Sci. 1 (1S): 4 – 8

Lee, S.H., Jung, B.Y., Rayamahji, N., Lee, H.S., Jeon, W.J., Choi, K.S., Kweon, C.H. and Yoo, H.S. 2009. A multiplex real time PCR for differential detection and quantification of *Salmonella* spp., *Salmonella* enterica serovar Typhimurium and Enteritidis in meats. J. Vet.Sci. 10:43-51

Moussa, I.M., Ashgan, M.H., Mahmoud, M.H. and Al-Doss, A.A. 2012. Rapid detection and characterization of *Salmonella* enterica serovars by multiplex polymerase chain reaction assay. Afr. J. Biotechnol. 11(14):3452-3458

Pinto, B., Chenoll, E. and Aznar, R. 2005. Identification and typing of foodborne *Staphylococcus aureus* by PCR-based techniques. Syst. Appl. Microbiol. 28: 340–352

Rahimi, E., Nonahal, F. and Salehi, E.A. 2013. Detection of classical enterotoxins of *Staphylococcus aureus* strains isolated from raw meat in Esfahan, Iran. Health Scope. 2(2):95-8

Rahn, K., De Grandis, S.A. and Clarke, R.C. 1992. Amplification of an inv A gene sequence of *Salmonella* Typhimurium by PCR as a specific method of detection of *Salmonella*. Molec. Cell. Probes. 6:271-279

Robles, G.M., Loredo, A., Ojeda, G., Vega, A., Chew, Y., Velarde, S. and Fratamico, P. 2008. Identification of *Salmonella* serovars isolated from Cantaloupe and Chile pepper production systems in Mexico by PCR-RFLP. J. Food Protect.71 (11): 2217-2222.

Santos, L.R., Nascimento, V.P., Oliveira, S.D., Flores, M.L., Pontes, A.P., Ribeiro, A.R., Salle, C.T.P. and Lopes, R.F.F. 2001. Polymerase chain reaction (PCR) for the detection of *Salmonella* in artificially inoculated chicken meat. Rev. Inst. Med. Trop. S. Paulo. 43(5):247-250

Xu, Y.G., Cui, L.C., Tian, C.Y., Li, S.L., Cao, J.J., Liu, Z.M. and Zhang, G.C. 2012. A multiplex polymerase chain reaction coupled with high performance liquid chromatography assay for simultaneous detection of six foodborne pathogens. Food Control. 25:778–783

Yemisi, O.A., Oyebode, T.A., Margaret, A.A. and Justina, B.J. 2011. Prevalence *of Arcobacter, Escherichia coli, Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. Sierra Leone J. Biomed. Res. 3 (1):8-12

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