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Short Communication

Detection of *Salmonella spp* contamination of carcasses slaughtered in poultry abattoir in Mashhad, Iran

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ABSTRACT

A total of 60 neck skin swab samples were taken from 12 different broiler flocks after the chilling stage of processing at a commercial broiler slughtering facility in Mashhad. The presence of *Salmonella* was assessed by conventional culture method and confirmed by using poly O and poly H antiserum in serotyping. PCR amplification of *invA* gene as a specific method for detection of *Salmonella* was evaluated. In this study *Salmonella* was isolated from 11.66% of samples by conventional culture method, then in serological test 28.6% of them detected as serogroup B and 71.4% as serogroup C. In this investigation all positive results by conventional culture method were confirmed by PCR amplification. Because of rapidity and high specificity and sesitivity of PCR method, by standardization of this method, it could be concidered as an alternative to conventional culture method for confirmation of *Salmonella* presence in raw poultry meat.

Keywords: Poultry meat, Salmonella, PCR, *invA* gene, conventional culture method

INTRODUCTION

Salmonella spp, cause one of the most important food borne disease in the world called *Salmonellosis* (Malorny *et al* 2003a). Foods with animal source such as beef, poultry meat, egg and milk have been shown to carry these pathogens (Gillespie *et al* 2003). Poultry products have been recognized as a major source of human contamination by these pathogens (Corry *et a*l 2002). It is reported that in addition to miss handling of poultry product and raw poultry carcasses, uncooked poultry meat is also one of the most frequent causes of human infection by *Salmonella* (Panisello *et al* 2000). Conventional culture methods, which use to isolate *Salmonella*, are including non-selective pre-enrichment follow by selective enrichment and plating on selective and differential agars. Then suspect colonies are confirmed by biochemical and serological methods (Van Kessel *et al* 2003, Manzano *et al* 1998). Generally these techniques take longer time, since they give only presumptive results after 3-4 days and definitive results after 5-6 days (Manzano *et al* 1998, Malorny *et al* 2003b). Although rapid detection methods, such as DNA or

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RNA probes, immuno-detection methods and nucleic acid hybridization have been developed, but they have not adequate sensitivity and specificity (Li *et al* 2000). In vitro amplification of DNA by the PCR is a powerful tool in microbiological diagnostics (Malorny$_b$ *et al* 2003). Several PCR methods for detecting *Salmonella* strains have been introduced. They utilize specific gene sequences for targeting (Manzano *et al* 1998). In 2003 as a part of the major international research project for the validation and standardization of PCR for the detection of five major food borne pathogens including *salmonella*, the most selective primer set was found to be 139-141, which targets the *invA* gene, this set of primers were published previously (Rahn *et al* 1992). This specific PCR assay, which was validated in this project, showed high selectivity on 242 *Salmonella* strains (inclusivity 99.6%) and 122 non-*Salmonella* strains (exclusivity 100%) (Malorny *et al* 2003a). The present study reports isolation of *Salmonella* from poultry carcasses by conventional culture method and confirmation of this result by PCR assay. This PCR assay can be used as a rapid detection method with high sensitivity and specificity which dose not need the long procedure in conventional culture method.

MATERIALS AND METHODS

Sample collection. After the chilling stage of the slaughtering process 60 swab samples were taken from the neck skin of broiler`s carcasses from 12 different flocks (5 sample from each flock) in one of the local slaughterhouse in Mashhad (north-west of Iran). Samples were obtained by use of wet and dry swabbing method (Vanderzant *et al* 1992) then they were placed in the tubes cotaining 5ml of 0.1% BPW(buffered pepton water) and transported to the laboratory for testing in crashed ice and analysed within four hours.

Conventional culture. Swabs which were located in 5ml of BPW were mixed with the same volume of double-concentrated lactose broth and incubated at 37 °C for 24 h, one milliliter of lactose broth were added to 9 ml of each tetrathionate broth and selenit cystine broth, followed by incubation at 42 °C and 37 °C respectivly for 24 h. A loopfull of each enriched culture were streaked onto BGA (brilliant green agar) and SS agar (*Salmonella Shigella* agar) and incubated at 37 °C for 24 h. Suspect colonies were confirmed biochemically by inoculating into lysine decarboxylase broth, urea broth and TSI agar slopes (triple sugar iron agar). Confirmation of presumptive posetive results performed by using of poly O and poly H antisera (Wellcolex colour *Salmonella*- Remel Inc).

PCR amplification. Suspected colonies from selective media were cultured on LB agar and incubted at 37 °C for 24 h, and then a loopful of bacteria was mixed with 300 µl of distilled water, and boiled for 10 minutes, then centrifuged at 6000rpm for 5 minutes. The amount of 1.5µl of supernatant was used for PCR amplification test using *Salmonella* specific primers based on the *invA* gene. The sequence of S139 and S141 primers was respectivly as follow: 5´- GTG AAA TTA TCG CCA CGT TCG GGC AA -3´ and 5´- TCA TCG CAC CGT CAA AGG AAC C -3´ (10). Reactions with these primers were carried out in a 25µl amplification mixture consisting of 2.5 µl 10x PCR buffer (500mM KCl, 200 mM Tris HCl), 1.25 µl dNTPs (10 mM), 1.6 µl Mg Cl_2, 0.5 µl of each primer, 0.5µl of Taq DNA polymerase (fermentase) and 1.5µl of boiled bacteria supernatant. Amplification was performed in thermocycler (Techneh, TC512). The cycling condition was as follow: an initial incubation at 94 °C for 60 seconds, followed by 35 cycles of denaturation at 94 °C for 60 seconds, annealing at 64 °C for 30 seconds, elongation at 72 °C for 30 seconds, and final extention priod for 7 minutes at 72 °C. Amplified products from *Salmonella* specific-PCR were electrophoresed in 1.2% agarose gel and a 100bp DNA ladder was used as a size reference. After

staining with ethidium bromide the gel was ducumented with a vidio camera. A positive response was defined by the presence of a visible band at the expected size. Deionised distilled water and *E. coli* O2 K12 were used as negative control and *S. typhymurium* (ATCC: 25923) were used as posetive control.

RESULTS AND DISCUSSION

A total of 60 samples were analysed by conventional culture method for the detection of *Salmonella*. Based on this method 7 samples (11.66%) were found positive and in serological test two samples were detected as serogroup B (28.6%) and five samples as serogroup C (71.4%). In PCR assay, using S139 and S141 primers that amplifies a 284 bp sequence of the *invA* gene, all positive samples in conventional culture method, generated a 284 bp amplified DNA fragment (Figure 1).

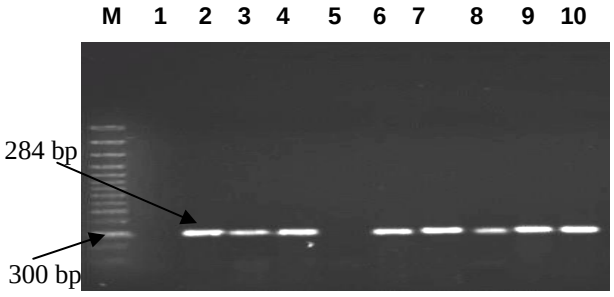

Figure1. Electrophoretic analysis of 284 bp amplification product from *invA* gene of *Salmonella spp* Isolated from raw poultry meat. M: 100bp marker. *Lane*1: negative control (water) - 5: negative control (E. coliO2 K12). 2: positive control (*S. typhymurium* ATCC: 25923). *Lanes 3, 4, 6, 7, 8, 9, 10*: samples.

Prevalence of *Salmonella* spp, has been investigated in poultry carcasses in Iran (Shiraz), using specific primers for *invA* gene in PCR method (Zahraei Salehi *et al* 2005).

Culture techniques are universally recognised as the standard methods for detection of bacterial pathogens, such as *Salmonella* in food stuffs (White *et al* 2002). These techniques generally take longer time, because obtaining definitive results take 5-6 days (Manzano *et al* 1998, Malorny *et al* 2003b). Background microorganisms in the food matrix can negatively influence the growth of the target pathogen (Bulte *et al* 1995). The degree and type of sublethaly injured or stressed *Salmonella* cells can influence the bacterial recovery and consequently limiting detection of this pathogen (Malorny *et al* 2003b). The presence of growth inhibitory factors in the selective media may also limit the growth of *Salmonella* on the laboratory media (White *et al* 2002). In this study we used preenrichment and enrichment stage for recovery of injured or stressed bacteria. It has been reported that higher sensitivity of PCR methods compare to culture techniques can be use for the detection of *Salmonella* (Gibson 1998). PCR assay for the detection of *Salmonella* which was evaluated in an international collaborative study, showed an analytical accuracy of 98% **(**Malorny *et al* 2003a**).** Based on these studies, the PCR based methods can be an alternative to the traditional culture method (Malorny *et al* 2003b).

Some PCR amplification methods for detection of *Salmonella* has been recommended such as amplification of *fimA* gene (Cohen *et al* 1996) , *16s rRNA* gene (Lin *et al* 2004), *agfA* gene (Hashimoto *et al* 1995), virulence associated plasmid (Rexach *et al* 1994), *viaB* gene (Hashimoto *et al* 1995), and *sefA* gene (Medici *et al* 2003). It has been suggested that the most selective primer set, uses the *invA* gene as the target (Oliveira *et al* 2002, Malorny *et al* 2003b). In this study we used primers targeting the *invA* gene of *Salmonella* to confirm the isolated colonies. Although in this study all of the isolated colonies were also posetive in PCR amplification. But it has been reported that some strains of *Salmonella* such as *Salmonella litchfield* and *Salmonella senftenberg* could not be detected by these primers (Rahn *et al* 1992). In addition some

Salmonella spicies also could not be detected by other PCR methods (Van Kessel *et al* 2003). It is obvious that the use of either conventional or PCR method in isolation and identification would have resulted in the failure to detect *Salmonella* in a number of positive samples (White *et al* 2002). Therefore, it may be pertinent to use a combination of the PCR and conventional culture methods in order to maximise the detection of *Salmonella* in naturally contaminated poultry carcasses. In this study we used PCR method as an alternative to conventional confirmation methods.

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References

Bulte, M. and Jakob, P. (1995). The use of a PCR-generated invA probe for the detection of Salmonella spp. In artificially and naturally contaminated food. *International Journal of Food Microbiology* 26 (3): 335-344.

Cohen, H.J., Mechanda, S.M. and Lin, W. (1996). PCR amplification of the fimA gene sequence of Salmonella typhymurium, a specific method for detection of salmonella. *Applied Enviromental Microbiology* 62 (12): 4303-4308.

Corry, J.E.L., Allen, V.M., Hudson, W.R., Bresline, M.F. and Davies, R.H. (2002). Source of salmonella on broiler carcasses during transportation and processing: modes of contamination and method of control. *Applied Microbiology* 92: 424-432.

Gibson, D.M. (1998). Rapid and definitive detection of *Salmonella* in foods by PCR. *Letters in Applied Micobioogy* 26: 437-441.

Gillespie, B.E., Mathew, A.G., Draughon, F.A., Jayarao, B.M. and Oliver, S.P. (2003). Detection of *Salmonella* enterica somatic groups C1 and E1 by PCR-enzyme-linked immunosorbent assay. *Journal of Food Protection* 66 (12): 2367-2370.

Hashimoto, Y., Itho, Y., Fujinaga, Y., Khan, A.Q., Sultan, F., Miyake, M., Hirose, K., Yamamoto, H. and Ezaki, T. (1995). Development of nested PCR based on the ViaB sequence to detect *Salmonella typhimurium. Journal of Clinical Microbiology* 33: 775-777.

Li, X., Boudjellab, N. and Zhao, X. (2000). Combined PCR and slot blot assay for detection of *Salmonella* and *Listeria monocytogenes*. *International Journal of Food Microbiology* 56: 167-177.

Lin, C.K., Hung, C.L., Tsai, C. and Tsen, H.Y. (2004). An improved PCR primer pair based on 16S rDNA for the specific detection of *Salmonella., Journal Food Protection* 67 (7): 1335-1343.

Malorny, B., Hoorfar, J., Bunge, C. and Helmuth, R. (2003a). Multicenter Validation of the Analytic Accuracy of *Salmonella* PCR: toward an international standard. *Applied Environmental Microbiology* 69 (1): 290-296.

Malorny, B., Hoorfar, J., Hugas, M., Heuvelink, A., Fach, P., Ellerbyoek, L., Bunge, C., Dorn, C. and Helmuth, R. (2003b). Interlaboratory diagnostic accuracy of a *Salmonella* specific PCR-based method. *International Journal of Food Microbiology* 89: 241-249.

Manzano, M., Coclin, L., Astori, G., Pipan, C., Botta, G.A., Cantoni, C. and Comi, G. (1998). Development of a PCR microplate hybridization method for simple, fast and sensitive detection of *Salmonella* serovars in food. *Molecular and Cellular Probes* 12: 227-234.

Medici, D.D., Croci, L., Delibato, E., Pasquale, S.D., Filetici, E. and Toti, L. (2003). Evaluation of DNA extraction methods for use in combination with SYBR Green I real-time PCR to detect *Salmonella* enterica Serotype enteritidis in poultry. *Applied Environmental Microbiology* 69: 3456–3461.

Oliveira, S.D., Santos, L.R.D., Schuch, M.T., Silva, A.B.C., Salle, T.P. and Canal, C.W. (2002). Detection and identification of salmonellas from poultry-related samples by PCR. *Veterinary Microbiology* 87: 25-35

Panisello, P.J., Rooney, R., quantick, P.C. and Stanwell-Smith, R. (2000). Application of foodborne disease outbreak data in the development and maintenance of HACCP system. *International Journal of Food Microbiology* 59: 221-234.

Rahn, K., DeGrandis, S., Clarke, R. and Mcewen, S. (1992). Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and Cellular Probes* 6 (4): 271-279.

Rexach, L., Dilasser, F. and Fach, P. (1994). Polymerase chain reaction for Salmonella virulence-associated

plasmid genes detection: a new tool in *Salmonella* epidemiology. *Epidemiology Infection* 112: 33-43.

Vanderzant, C. and Splittstoesse, D.F. (1992). *Compendium of Methods for the Microbiological Examination of foods*. (3rd edn,). Pp: 57-59. American Public Health Association.

Van Kessel, J.S., Karns, J.S., Perdue, M.L. (2003). Using a portable real-time PCR assay to detect Salmonella in raw milk. *Journal of food protection* 66: 1762-1767.

White, P., Meglli, K., Collins, D. and Gormely, E. (2002). The prevalence and PCR detection of *salmonella* contamination in raw poultry. *Veterinary Microbiology* 89 (1): 53-60.

Zahraei-Salehi, T., Mahzounieh, M., Saeedzadeh, A (2005). Detection of *InvA* gene in isolated *Salmonella* from broilers by PCR method**.** *International Journal of Poultry Science* 4 (8): 557-559.