SALMONELLA SPP. ISOLATION FROM CHICKEN SAMPLES AND IDENTIFICATION BY POLYMERASE CHAIN REACTION

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Summary


In this study, carcass, intestine, liver, gall bladder and spleen samples of chickens slaughtered at an abattoir in the Elazig province were tested for the presence of Salmonella spp. The identity of Salmonella spp. was further proved by culture and by polymerase chain reaction (PCR). A total of 1250 samples were tested. All samples were collected from different commercially reared chicken flocks. Chicken carcasses, intestines, livers, gall bladders and spleen were found positive with proportions of 12%, 7.2%, 4%, 2% and 1.6% respectively, by both culture and PCR. The Salmonella detection rate was the highest (12%) in chicken carcasses and the lowest (1.6%) in spleen.

This study showed that Salmonella spp. was widespread among the chicken population in Elazig.

Key words: chicken, culture, PCR, Salmonella spp.

INTRODUCTION

Salmonella is one of the most important pathogens responsible for human food poisoning in the developed world (Cerro et al., 2002) and chicken products are widely acknowledged to be a significant reservoir for Salmonella. They have frequently been incriminated as a source of salmonellae contamination and consequently thought to be major sources of the pathogen in humans (Uyttendaele et al., 1998; Baeumler et al., 2000). Furthermore, one of the commonest causes of Salmonella infection reported in humans has been through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat (Panisello et al., 2000). The number of reported cases of salmonellosis has increased about threefold in the United States during the past 20 years (Tauxe, 1997).

Standard culture methods for detecting Salmonella spp. in poultry include non-selective pre-enrichment followed by selective enrichment and plating on selective and differential agars (Whyte et al., 2002). These methods take approximately 4-7 days (Harvey & Price, 1979; Perales & Audicana, 1989). Since Salmonella is closely related to both public and animal health, more rapid and sensitive methods for the identification of this bacterium are
Salmonella spp. isolation from chicken samples and identification by polymerase chain reaction

required (Schrank et al., 2001). Several alternative, faster methods for the detection of Salmonella have been developed, the use of the polymerase chain reaction (PCR) being one of the most promising approaches (Candrian, 1995; Scheu et al., 1998).

The aim of this study was to estimate the prevalence of Salmonella spp. in chicken carcasses, intestines, livers, gall bladders and spleen, and confirmation of conventional identification using by PCR identification.

MATERIALS AND METHODS

Samples
Carcass, intestine, liver, gall bladder and spleen samples were collected from 250 commercially reared chicken slaughtered at an abattoir in the Elazig province located in Eastern Turkey. A total of 1250 samples were examined. The samples were immediately transported to the laboratories in a cool thermos and were processed for culture.

Cultures
Salmonella was isolated according to standard methods (ISO 6579, 1993). Twenty five g sample of chicken carcass was added to 225 mL of buffered peptone water (BPW, Oxoid, Basingstoke, UK). One g of intestine, liver, gall bladder and spleen were aseptically added to 9 mL of the same preenrichment medium. All samples were incubated for 18 h at 37°C, respectively. After 24 and 48 h of incubation respectively, one loopful from each of the enriched broths was streaked onto plates of Salmonella Shigella (SS) agar (Difco) and xylose lysine deoxycholate (XLD) agar (Difco) and incubated at 37°C for 24 h. The plates were examined for the presence of typical colonies of Salmonella, i.e. transparent colonies with black centres on SS agar and red colonies with black centres on XLD agar (Antunes et al., 2003). Suspected colonies were confirmed by conventional biochemical methods (Lautrop et al., 1979; Nissen, 1984).

DNA extraction
A few colonies growth on selective agar was transferred into an Eppendorf tube containing 300 µL sterile distilled water. The tubes were vortexed and incubated at 56°C for 30 min. The suspension was then added in to 300 µL of TNES buffer (20 mM Tris pH 8.0 + 150 mM NaCl + 10 mM EDTA + 0.2 % SDS) and 200 µg/mL Proteinase K. Following 30 min boiling, an equal volume of phenol was added to the suspension which was shaken vigorously by hand for 5 min and then, centrifuged at 11600 x g for 10 min. The upper phase was transferred into a new Eppendorf tube. DNA was precipitated with absolute ethanol and 0.3 M sodium acetate at –20°C for 1–2 h. The mixture was then centrifuged at 11600 x g for 10 min and the upper phase was removed. The pellet was washed twice with 90% and 70% ethanol, respectively and each step was centrifuged at 11600 x g for 5 min. Finally, the pellet was dried, resuspended in 50 µL sterile distilled water, and stored at –20 °C until further use (Cetinkaya et al., 2002).

Reference Salmonella enteritidis strain
(Kindly provided by Dr. A. A. Mohamed)
Hatha, Department of Biology, The University of the South Pacific, Private Mail Bag, Suva, FIJI) was used in PCR tests as a positive control.

Primers

The primers used were: 16SF1 (5’-TGTTGTTGTTAATAACCGCA-3’) and 16SIII (5’-CACAAATCCATCTCTGGA-3’) (Promega) derived from 16S rRNA gene (Lin & Tsen, 1996).

PCR

The reaction mixture was prepared in a total volume of 50 µL containing 5 µL of 10x PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 5 µL of 25 mM MgCl2, 250 µM of each deoxynucleoside triphosphate, 2 U of Taq DNA Polymerase (Fermentas, Lithuania), 10 pg of each primers and 5 µL samples of extracted bacterial DNA. PCR involved 35 cycles of denaturation (94°C, 1 min), primer annealing (58°C, 1 min) and primer extension (72°C, 1 s). The primer extension step (72°C, 10 min) followed the final amplification cycle (Fluit et al., 1993). For all experiments, a Touchdown Thermocycler (Hybaid, Middlesex, England) was used. PCR reaction products (15 µL) were analysed by electrophoretic separation on 1.5% agarose gels stained with ethidium bromide. The gel was visualized by UV illumination and photographed with Polaroid films.

RESULTS AND DISCUSSION

Suspected isolates were biochemically identified as Salmonella spp. (Lautrop et al., 1979; Nissen, 1984). In 12%, 7.2%, 4%, 2% and 1.6% of the samples of chicken carcasses, intestines, livers, gall bladders and spleen, respectively Salmonella spp. were isolated (Table 1).

In the PCR examination, positive results with the the molecular size of 572-bp were obtained from all Salmonella spp. suspicious isolates (Fig. 1).

The need for the development of rapid and accurate detection methods for Salmonella spp. has been increased in recent years due to the higher incidence of salmonellosis in industrialized countries over the past decades (Tauxe, 1991; Lewis, 1997) since the conventional methods for the isolation and identification of salmonellae require up to 4-7 days. Recently, the PCR has become a powerful and increasingly popular tool in microbial identification (Persing, 1991).

The primers 16SF1 and 16SIII were proved to be specific for the PCR detection of all Salmonella isolates with various serogroups (Lin & Tsen, 1996). For these reasons, we used the primers 16SF1

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of Salmonella spp.</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen (250)</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>Gall bladder (250)</td>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td>Liver (250)</td>
<td>10</td>
<td>4.0</td>
</tr>
<tr>
<td>Intestine (250)</td>
<td>18</td>
<td>7.2</td>
</tr>
<tr>
<td>Carcass (250)</td>
<td>30</td>
<td>12.0</td>
</tr>
</tbody>
</table>
and 16SII derived from the 16S rRNA gene and found that all Salmonella isolates identified by conventional tests gave positive bands with PCR.

Up to 70% of broiler carcasses are contaminated with Salmonella (Mead, 1982). Our findings of contamination rates with Salmonella were lower than those observed in the other countries, as 65.4% in the USA (Waltman et al., 1992), 57% in Portugal (Machado & Bernardo, 1990) but higher than 1.2% in Scotland (Brown et al., 1973). A study made between January 2000 and July 2001 and from July 2001 until December 2003 by Eyigor et al. (2005) revealed that Salmonella organisms are detected in 4.10% and 5.52% of chicken samples, respectively. When comparing our results to those of other studies performed in Turkey, our data of the prevalence of Salmonella spp. from chicken were lower than the results (69.77% and 24%) in Bursa and Istanbul (Carli, 1990; Ang-Kuçuker et al., 1993), were in agreement with the results (11.4% and 10.81%) in Ankara and Elazig respectively (Ugur, 1992, Kalender & Muz, 1999), but higher than data (4.2%) reported in Konya (Orhan & Guler, 1993) and by Eyigor et al. (2005).

Studies of meat and poultry products revealed salmonellae in Canada for the period 1983–1986 in 60.9% of 670 chicken samples (Lammerding et al., 1988); in the USA for the period 1994–1995 in 20% of 1297 broiler carcasses (United States Department of Agriculture, 1996). In a Spanish study on 192 chicken livers and carcasses, 80% and 60% of samples, respectively were positive for Salmonella (Carraminana et al., 1997). In Venezuela, 41 out of 45 chicken carcasses studied yielded salmonellae (Rengel & Mendoza, 1984).

In this study, the Salmonella detection rate was the highest (12%) in chicken carcasses. This finding is in agreement with the results of Capita et al. (2003) reporting that contamination rates of chicken carcasses (55%) were higher than those of chicken parts (wings, legs, livers and hearts) (40%).

When comparing our results to those of other authors, several factors must be taken into consideration, such as differences in origin, time period and age of the samples, sampling procedure, contamin-

Fig. 1. An agarose gel stained with ethidium bromide, with PCR products of Salmonella isolates (M: 100 bp DNA ladder, P: positive control, 1-13: Salmonella isolates).
tion level of animals, slaughterhouse sanitation, level of processing and cross-contamination of the products, and differences in methodology applied to detect the pathogen (Bryan & Doyle, 1995; Uyttendaele et al., 1999).

A study on the prevalence of Salmonella spp. in organs of chickens have reported that 8.91%, 5.31%, 3.42% and 3.23% Salmonella spp. were isolated from intestines, livers, gall bladders and spleen, respectively (Kalender & Muz, 1999). In this study, the highest isolation percentage (7.2%) was obtained from the intestines among all chicken organs examined. This proportion is in agreement with the results (8.91%) reported by Kalender & Muz (1999). This may be due especially to the intestinal agents of paratyphoid.

This study showed that Salmonella spp. was widespread among the chicken carcasses and internal organs of slaughtered chickens in Elazig. It may be due to insufficient hygiene, during slaughtering and processing of the flocks in the region. Further studies are needed to improve surveillance strategies to decrease the prevalence of Salmonella spp. in chicken population of Elazig.

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