Detection of Virulence Gene (invA) in Salmonella Isolated from Meat and Poultry Products

Mohamed Karmi

Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University, 81528 Aswan, Egypt

Abstract: Detection of pathogenic Salmonella isolated from meat and poultry products by detecting virulence invA gene using PCR technique. A total of 100 meat and poultry products samples were collected from shops and supermarkets; including 50 meat products samples and 50 poultry products. Salmonella was detected by conventional isolation methods and then PCR was carried out to detect invA gene in isolated Salmonella. It was observed that 16% of samples were positive for Salmonella isolation; 26% (13/50) in meat samples and 6% (3/50) in poultry samples. All Salmonella isolates were positive for the invA gene. Meat and poultry products were contaminated with pathogenic Salmonella and all isolated Salmonella contained the virulence gene, invA gene, assuring that these isolates are pathogenic and virulent strains. Hygienic measures should be undertaken to reduce contamination of meat and poultry products with pathogenic Salmonella.

Key words: Salmonella • Virulence • invA gene • Meat • Poultry • Products

INTRODUCTION

In the last three decades, Salmonella enterica subtype Enteritidis has become one of the main agents causing food borne diseases worldwide with considerable economic impact. This agent is transmitted to humans mainly by contaminated meat and poultry products (88%), suggesting strong relationships between cases of Salmonella related to human illness and Salmonella positive in meat and poultry products [1-4]. In 2007 Austria reported a total of 438 food borne outbreaks, Salmonella caused 70% of the bacterial outbreaks and the most implicated food was the poultry meat products [6], while in rural area in Thailand, ninety-seven percent of food samples were contaminated with at least one enteric pathogen, Salmonella is one of the most commonly isolated pathogen about 84%, moreover, fifty-one percent of children in the same area infected with alike Salmonellaserotypes isolated from meat samples, suggesting this pathogen is widespread in food and humans [5].

One of the earliest steps in the pathogenic cycle of the facultative intracellular pathogen Salmonella spp. is the invasion of the cells of the intestinal epithelium. The ability of Salmonella strains to cause invasive disease is attributed to arrays of virulence genes defined in the Salmonella pathogenicity islands (SPIs) [14]. There are at least 60 genes associated with SPIs [15] and the majority of these determinants are located on the chromosome or on large virulence-associated plasmids [16, 17]. A genetic locus, inv, was identified that allows Salmonella spp. to enter cultured epithelial cells. invA is a member of this locus and it is the first gene of an operon consisting of at least two additional invasion genes [7].

Established conventional methods to detect and identify Salmonella are time consuming and include selective enrichment and plating followed by biochemical tests [9, 10]. On the other hand, polymerase chain Reaction (PCR) is a rapid and reliable method for detection and identification of food borne pathogens such as Salmonella as a complementary to conventional culture[11, 12]. Several genes have been used to detect Salmonella in natural environmental samples as well as food and faecal samples[19]. Virulence chromosomal genes including; invA [46, 47], invE [48], himA [49], phoP [50]. The invA gene of Salmonella contains sequences unique to this genus and has been proved as a suitable PCR target with potential diagnostic application [20].
Enrichment-PCR on the basis of invA gene was simple, rapid, sensitive and specific in comparison with traditional method. It could be applied in fast diagnosis of food poisoning and actual food samples screening [13].

Five major SPIs are well characterized in SalmonellaTyphimurium and SalmonellaTyphi, namely, SPI1, SPI2, SPI3, SPI4 and SPI5 and these SPIs are the basis of pathogenicity in the host [18]. The multiplex real-time assay is a simple, rapid, inexpensive, reliable system and can be used as a valuable tool to detect virulence invasion gene-carrying Salmonella, thus enhancing the safety of food [21, 22]. Application of pyrosequencing of six Salmonella-specific genes as aapid Salmonella identification method was tested. Primers for hns, hisJ and hilA had non-specific reactions with non-Salmonella strains. Primers for invA, iroB and filmy had specific PCR products and pyrosequences of Salmonella, suggesting that they were suitable for Salmonella rapid identification [23].

In this study we investigate the presence of virulence gene (invA) in strains of Salmonellae isolated from meat and poultry products.

MATERIALS AND METHODS

Collection and Preparation of Samples: A total of 100 meat and poultry products samples (50 each) were collected from shops and supermarkets. Samples were collected in sterile polyethylene bags, put in ice tank under low temperature and transported to the laboratory for bacteriological examination. Twenty-five gramsof each sample was taken in sterile stomacher bag, mixed with 225 mlbuffered peptone water (BPW) (Oxoid Limited, Hampshire, England)and homogenized by using Stomacher® 400 Circulator (Seward Ltd. UK).

Isolation and Identification: The samples mixtures incubated at 37 ± 2 °C for 18 ± 0.2 h, 0.1 ml mixture was transferred to 10 ml Rappaport-Vassiliadis (RV) medium, vortexed and incubated for 24 ± 2 h at 42 ± 0.2°C (circularing, thermostatically-controlled, water bath). Three millimetre loopfull (10 µl) of each RV tube was streaked on Xylose Lysine Desoxycholate (XLD) agar and incubated for 24 ± 2 h at 35°C. Typical colonies of Salmonellawere pink colonies with or without black centers. Many cultures of Salmonella may produce colonies with large, glossy black centers or may appear as almost completely black colonies. Salmonella isolates were confirmed by biochemical tests as Triple Sugar Iron agar (TSI), Lysine decarboxylase (LIA), Urease, Indole, Methyl red, Voges-Proskauer and Simmons citrate and serological agglutination with somatic (O) and flagellar (H) antigens [24-28].

DNA Extraction of Isolated Salmonella: Bacteria were cultured on LB agar for 24 hours at 37°C. Extraction of DNA was performed by suspending pellet in 200 µLTE buffer [10 mmol/L Tris-HCl (pH 8.0), 1 mmol/LEDTA] and the mixture was briefly mixed on a vortexmixer. The suspension was placed in a boiling water bath for 1 min, subjected to 3 freeze-thaw cycles alternating between -70°C for 3 min and 100°C for 2 min, for 10 minutes and centrifuged at 6000 rpm for 5 minutes; the pellet was then washed 3 times by suspending it in 1.5 mL acetone. The supernatant were used for amplification by PCR with Salmonella specific primers [8, 51].

Primers Set and Pcr Amplification Program: Salmonella specific primers S139 and S141 Rahn et al. [20] have respectively the following nucleotide sequence based on the invA gene of Salmonella 5'- GTG AAA TTA TCG CCA CGT TCG GGC AA - 3’ and 5’- TCA TCG CAC CGT CAA AGG AAC C - 3’. Reaction with these primers were carried out in a 25 µl PCR mixture consisting of 2.5 µl 10X PCR buffer (500 mMKCl, 200 mMTrisHCl), 1.25 µl dNTPs (10 mM), 1.6 µl MgCl2 0.5 µl of each primer, 0.5 µl of Taq DNA polymerase and 1.5 µl DNA template of each isolate were used in the reaction. Amplification was conducted in Master-gradient Thermocycler (Eppendorf). The cycle conditions were 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 and elongation at 72°C, followed by 7 minutes final extension period at 72°C.

Electrophoresis of PCR Products: The amplified DNA products from Salmonella specific-PCR were analysed with electrophoresis on 1.2 % agarose w/v gels stained with ethidium bromide and visualized by UV illumination. A current of 120 V was applied to each gel. Eight microliter of PCR product mixed with 3 µl of 6X loading dye was loaded on to agarose gel. A 100 bp DNA ladder was used as a marker for PCR products.

RESULTS

It was observed that 16 % of samples were positive for Salmonella; 26% (13/50) in meat samples and 6% (3/50) in poultry samples. The identified serotypes of isolated Salmonella were Salmonella enteritidis 6.25% (1/16), ...
Salmonella fortune 12.5% (2/16), Salmonella agona 6.25% (1/16), Salmonella hessarek 6.25% (1/16) and Salmonella hoboken [32]. The presence of invA gene was expanded the host range of Salmonella spp. [40]. Some studies confirmed that invasion gene(invA), secreted effector protein gene (sipA) other gene fragments are carried on Salmonella pathogenicity island 1 (SPI-1) and Salmonella strains lacking this SPI-1 have invasiveness rates towards Hela cells significantly lower than the rate of invA-positive strain STM1344[41]. Some genes encoding for virulence factors located on plasmids, can be transferred from one strain to another and can cause an increase in the pathogenicity of serotypes [42]. Different serotypes can be present in different animal species: some of them are considered species-specific, while others are ubiquitous [43]. The presence of virulence plasmids in host-adapted serovars suggests that horizontal virulence acquisition can have expanded the host range of Salmonella [44]. In detection study 46 Salmonella strains were isolated from 42 meat samples; the positive rate was 20.1%. The majority of positive samples were fresh meat, 69.23% in duck, 37.14% in chicken, 20% in beef and 16.67% in pork. Application of hygienic approaches and effectiveness of potential interventions during production, slaughtering,
manufacturing, preparation and processing of meat products can significantly reducing the number of *Salmonella* positive samples in meat products [45].

**CONCLUSION**

Meat and poultry products were contaminated with pathogenic *Salmonella* and all *Salmonella* isolates are positive for the presence of virulence gene (*invA*) that responsible for cell invasion. Meat and poultry products considered an important source of high risk serotypes of *Salmonella* for human, subsequently, hygienic measures should be undertaken to reduce contamination of meat and poultry products with virulent strains of *Salmonella* that is of public health significance.

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**REFERENCES**


