Molecular detection of *invA* gene for *Salmonella* spp. isolates from poultry in Babil province-Iraq

Safaa Jabbar Hamzah* Nafea Sabeeh Jasim Alaa Abdel-Kadhim Jawad
Coll. of Vet. Med. / Univ. of Al-Qadisiyah
*E-mail: Nafea.Jasim@qu.edu.iq **E-mail: ahmed.neamah@qu.edu.iq
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Abstract

A total of 150 poultry samples (age from 1 to 49 day) were collected from different locations at Babylon province (Al-hashimiya , Al-madhatiya and Al-Qasim) from November 2015 to April 2016. These samples were collected from different part of the body (Liver tissue, Yolk sac content, and cecal swab). *Salmonella* spp. was isolated and identified using bacterial culturing on selective media, in addition to, biochemical and Mini API 20E and serotyping by monovalent antisera. Polymerase chain reaction (PCR) was used to detect *invA* of *Salmonella* spp. The results revealed that the rate of *Salmonella* isolates from poultry specimens were (11) 7.3% using cultural and biochemical tests, the results of serotyping revealed these isolates belong to *Salmonella* spp. The PCR technique was used to detect *invA* gene, these *Salmonella* isolates appeared to contain this gene since DNA amplification showed one distinct band (size 389 bp) when electrophoresed on agarose gel. The results of this study revealed that the PCR technique had a high specific in detection of *Salmonella* spp. When compared with other conventional detection methods.

Keywords: Poultry, *Salmonella*, PCR, *inv*A gene

Introduction:

Salmonellae is a Gram-negative bacterium comprising two species, *S. enterica* and *S. bongori*, which encompass more than 2,600 serovars that are capable of causing infection in a wide range of hosts [1,2]. *Salmonella* represents a significant public health concern worldwide, commercial poultry are often persistently infected with non-typhoidal serovars of *Salmonella enterica*[3].The non typhoidal serovars of *Salmonella enterica* are a leading cause of invasive bacterial gastroenteritis in humans [4]. *Salmonella* Typhimurium and *Salmonella enteritidis* are the most common serovars associated with invasive nontyphoidal salmonellosis [5]. The use of selective and differential plating media is a simple method for the isolation of *Salmonella* spp., a wide variety of selective and differential media has been developed for this purpose, including xylose lysine desoxycholate agar (XLD), Hektoen Enteric (HE) agar, and bismuth sulfite (BS) agar [6].These traditional methods are generally time-consuming and the diagnosis may persist for 5-7 days, therefore we can reduce the time of diagnosis by using molecular technique. Polymerase chain reaction (PCR) which become alternative diagnostic method it's sensitive, fast and accurate. The *invA* gene contains sequences unique for detection *S.Typhimurium and S.Enteritidis* directly and give result within 12 hours [7]. The aim of this study was to evaluate the rapid detection Method for identification of salmonellosis by using molecular technique for the detection of *invA* gene of *Salmonella enterica*, and to compare the PCR with conventional methods.

Materials and Methods:

Samples Collection: A total of 150 poultry samples (age from 1 to 49 day) were collected from different locations at Babylon province(Alhashimiya,Almadhatiya and Al-Qasim) from different part of the body (Liver tissue, Yolk sac content, and Cecal swab).

Isolation and Identification of *Salmonella* spp.: The sample was placed on nonselective enrichment in Buffered peptone water (BPW), then culturing on tetrathionet broth overnight then a loop full of broth was streaked on surface of S.S, XLD and B.G agar plates and then incubated at 37C˚ for 24 hrs. The biochemical characters of non - lactose fermenting bacteria was determined by using TSI agar and Urease test and other

biochemical tests and serotyping this method will take 5-7 days [8]., and we can reduce time for 12 hours for 6 hours in BPW followed by cell breaking and PCR in 6 hours allows the detection of *Salmonella* spp.,[7].This study was conducted during the period that extended from November 2015 to April 2016.

CR method:

DNA Extraction and Purification: The DNA of all isolates were extracted and purified using genome DNA purification kit provided by Anatolia, Turkish).

Primers: Specific primers used for the detection specific sequence of *invA* gene for detection *Salmonella* spp.[7], which is provided by Alpha DNA company (Canada).

Table (1): Specific primers used for the detection specific sequence of *invA* gene

Sequence	Orientation	Product (bp)
5-GCTGCGCGCGAACGGCGAAG-3	forward	389 bp
5-TCCCGGCAGAGTTCCCATT-3	reverse	

These primers were prepared according to the information of company by dissolve each primer in 1000 µl of deionized distilled water to obtain stocks in concentration 12 picomol / µl of each of the PCR primers.

The *invA* Gene Detection:

For the detection of *Salmonella* spp. by PCR the specific primers of *invA* gene were used. The PCR amplification mixture (25µl) which was used for the detection *invA* gene includes 12.5 µl of (Green master mix, 2x, which provided by Kapabiosystem, South Africa) include: bacterially derived Taq DNA polymerase; dNTPs which include: 400 µM of each dATP, dGTP, dCTP, dTTP; 3mM of Mgcl2; Yellow and blue dyes as loading dye), 2.5 µl of template DNA, 1.25 µl of each forwarded and reversed primers and 7.5 µl of nuclease free water to complete the amplification mixture to 25 µl. The PCR tubes containing amplification mixture were transferred to thermocycler and started the program as in the following reaction: 95°C for 5min as initial denaturation then 35 cycles each cycle consisted of the following: 95°C for 90 sec (denaturation), 62°C for 60 sec (annealing), and 72°C for 90 sec (extension), and final extension 72°C for 420 sec .After PCR, the profiles of amplification products were detected by gel electrophoresis. Ten microliters of total reaction mixture was loaded on a 2% agarose gel and electrophoresed at 100V at 70 mA for 45 to 60 min. The amplicon were visualized by UV illumination after agarose gel electrophoresis and ethidium bromide staining by standard procedures.

Statistical Analysis:

All results were performed by Chi square test at the level of significant when P-value < 0.01.

Results:

Culture and biochemical tests:

Incidence of *Salmonella* according to sample types:

A total of 150 different types of samples have been investigated in this study, 50 samples were collected from liver, yolk sac and cecal swab. Out of these 150 studied samples, 11 were *Salmonella* positive (6%). Table (2) below indicated that significant differences (P<0.01) were found among different types of collected samples. However, cecal swabs (12%), and liver (8%) exhibited high percentage of isolation, where as yolk sac was (2%).

Table (2): Isolation rates of *Salmonella* spp. from collected samples.

Source of sample	No. of examined samples	No. of positive samples	Percentage of +ve samples
Cecal Swab	50	6	12
Liver	50	4	8
Yolk sac	50	1	2
Total	150	11	7.3
X^2 =28.269	df = 6		P < 0.01

***Salmonella* positive samples at different location of Babil province:**

A total number of 150 broiler samples which have been collected from different locations at Babil province indicated, as shown in table 4-3, that *Salmonella* detected at (6%) Al-Hashymia consisted of 3 isolates

out of 50 samples, while 5 out of 50 at (10%) Al- Madhatia, the table also showed at (6%) Al- Qasim 3 out of 50. Higher significant differences (P<0.01) were present among the studied locations.

Table (3): Prevalence of *Salmonella* spp. in broiler samples according to geographic area.

Location	No. of examined samples	No. of positive samples	% of positive samples
Al-Hashymia	50	3	6
Al-Madhatia	50	5	10
Al- Qasim	50	3	6
Total	150	11	7.33
X^2= 1.455	df = 2	P < 0.01	

PCR Results :

DNA Extraction :

The DNA of all isolates was extracted and purified using genome DNA purification kit. The results were detected by electrophoresis on 1% agarose gel and exposed to U.V light in which the DNA appeared as compact bands.

Amplification of target DNA (*invA* gene):

The results of PCR amplification which was performed on the DNA extracted from all the studied isolates were confirmed by electrophoresis analysis. By this analysis, the strands of DNA resulted from the successful binding between specific primers and isolates extracted DNA. These successful bindings appeared as single bands under the U.V light using ethidium bromide as a specific DNA stain. The electrophoresis was also used to estimate DNA weight depending on DNA marker (8000 bp DNA ladder) and the result of this estimation revealed that the amplified DNA of 389 bp (Figure 1).

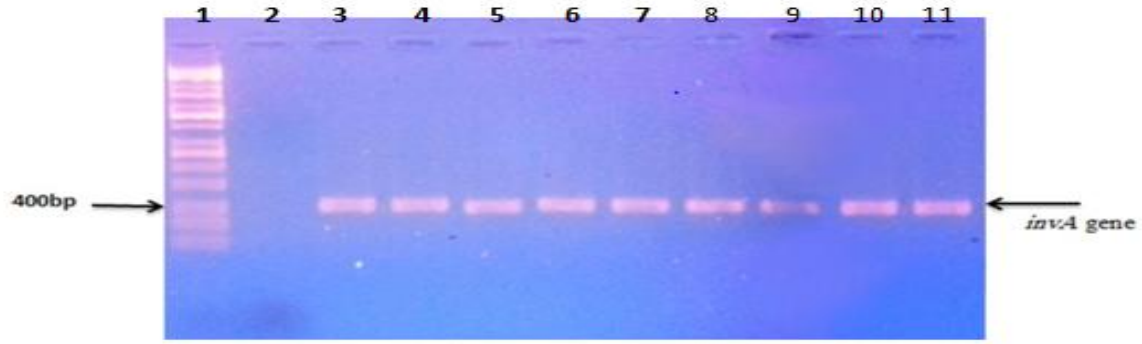

Fig .(1) DNA amplification of a 389 bp of *Salmonella* spp. detecting *invA* gene using PCR Lane1: ladder (8000bp), lane2: negative result, lane 3,4,5,6,7,8,9,101nd 11 positive results as *Salmonella* spp.

Discussions:

In this study, we found that *Salmonella* spp., in Babil province is considered one of the causes of salmonellosis in broiler. This may reflect the fact that *Salmonella* spp., is one of etiologic agents of diseases that that infect broiler in different ages and different seasons, and that *Salmonella* is a zoonotic bacterial agent, and *S. Typhimurium* is the most common serovar found in animals and humans infects mucosal gastrointestinal tract and causes a severe inflammatory diarrhea [(9)]. In this study, we found (11/150) 7.33% suspected isolates of *Salmonella* spp. on culture and biochemical tests. Traditional *Salmonella* detection methods are based on cultures using selective media and characterization of suspicious colonies by biochemical and serological tests by polyclonal and monoclonal antisera. Other studies revealed the prevalence of *Salmonella* spp. there were 7.9%, 8.47% respectively [(10, 11)]. *Salmonella* detection by using conventional media, such as *Salmonella-Shigella* agar (SS), is based on lactose fermentation and H_2S production. The number of false-positive results with these media necessitates time-consuming and expensive additional testing [(12)]. Because the conventional methods for *Salmonella* spp., have very poor specificity, and there were numerous false- positive results [(13)]. In this study, 11 isolates belong to *Salmonella* spp. (by using PCR detecting *invA* gene). The specific PCR product is an 389-bp fragment

which was visualized by gel electrophoresis and ethidium bromide staining. All *Salmonella* isolates gave positive results by the PCR, the *invA* gene contains sequences unique to *Salmonella* spp. isolates and demonstrate that this gene is a suitable PCR target for these isolates. While other study were used different target genes for detection Salmonella from different methods such as dection of *fimA, fimC ,hilA* genes which contains sequences unique to *Salmonella* isolates and demonstrate that this gene is a suitable PCR target for detection of *Salmonella* spp. [14,15,16].

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