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Detection of Salmonellae isolated from layer and broiler chickens samples by using Polymerase Chain Reaction Technique

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Abstract

In this investigation, 60 *Salmonella* isolates from poultry samples (liver,bile,spleen,heart, yolk sac,ceca, joint, ovary and oviduct) obtained from layer and broiler chicken were isolated in Iraq. The prevalence of *Salmonella* infection in studied samples was 10.4% . The bacteria were cultured, and biochemically characterized by biochemical test and the analytical profiling index (API 20E). The *inv*A gene primers were selected specifically for the detection of *Salmonella* to amplify a 284 bp DNA fragments by use polymerase chain reaction (PCR) assay . All *Salmonella* isolates were positive for the *inv*A gene. In order to provide a more accurate profile of the prevalence of *Salmonella*spp in layer and broiler samples, it is pertinent to use *inv* A gene specific PCR method that could be considered as an appropriate supporter or alternative to conventional culture method.

Keywords: *Salmonella*, detection, polymerase chain reaction, *inv* A gene

Introduction

The genus of *Salmonella* is a gram-negative rod shaped bacteria in the family of Entrobacteriacea. Poultry and poultry products have been implicated as a major source of *Salmonella* infections in human (Kumar, 2012). Avian salmonellosis is an important disease causing serious impediment to the development of poultry industry especially in developing countries of Asia and Africa (RajagopalandMini,2013).There are mainly two types of non motile avian *Salmonella* spp. namely *Salmonella gallinarum* and *Salmonella pullorum* that cause fowl typhoid and pullorum disease, respectively. Besides, motile Salmonellae (paratyphoid group) infection cause salmonellosis in chickens and have zoonotic significance (Hossain et al., 2006).

Conventional bacterial culture methods are still used most often to identify *Salmonella* and require at least 3-11 days. The standard culture methods for detecting *Salmonella* spp. in poultry include non-selective preenrichment followed by selective enrichment and plating on selective and differential agars .These methods are time consuming and labour intensive (Menghistu et al.,2011).Increased regulations and consumer demand for enhanced *Salmonella* testing in commercial poultry has created a need for high-throughput, fast and accurate alternatives to conventional culture methods which can take up to two weeks for a negative test on a delayed secondary enrichment test. PCR tests have demonstrated their utility as screening tools for *Salmonella* broiler and layer samples to reduce workloads and shorten

turnaround time for *Salmonella* testing (Bautista et al.,2011). The *inv*A gene of *Salmonella* contains sequences unique to this genus and has been proved as a suitable PCR target, with potential diagnostic applications (Shanmugasamy et al., 2011).

In this research, samples from layer and broiler chickens were tested, for isolation of *Salmonella*, by culturing and biochemical method and then they were confirmed by *inv*A specific PCR methods.

Materials and Methods

Sample collecting: A total of (577) samples were collected randomly from layer and broiler chicken farms (different ages) of Baghdad . The sample of each bird collecting aseptically, and put in sterile plastic container in cold box until reach to laboratory for culture.

Isolation of Salmonella: Samples were aseptically cultured into non selective broth (Peptone water broth) and incubated at 37^{o}C for 24 hours. then cultured on selective broth (Selenite F broth) (Himedia) and incubated at 37^{o}C for 24 hours. Subsequently, a loopful of each broth was streaked on surface of MacConkey agar plates (Oxoid), Xylose Lysine Desoxycholate agar (Himedia), Salmonella and Shigella agar (Himedia), Brilliant green agar (LAB) and chromogenic agar (Conda)for further incubation at 37^{o}C for 24 hrs(OIE.,2012).

Gram's staining : The isolated bacteria were stained by Gram's stain (according to manufacture of gram's stain kit) to determine their staining characteristics and purity of the culture.

Carbohydrate fermentation test and biochemical test: Basic sugars such as glucose, sucrose, lactose, mannitol, dextrose and maltose were used for sugar fermentation test. Biochemical tests included oxidase, catalase, urease, TSI, indole, lysine iron agar and simmone's citrate test (Quinne et al., 2004). Further confirmation of the presumptive Salmonella isolates was carried out with a commercial bacterial identification kit such as the Analytical Profile Index (API) system (Liofilchem, Italy).

Molecular identification: The DNA from the enriched culture was obtained by using a DNA extraction kit (Genaid, Korea) and the purified DNA was used as a template for the PCR assay. For the PCR, the sequence of primer used in this study is shown in (Table 1).

(Table 1) : Sequence of primer used for detection of *Salmonella* spp

No	Primer Sequences	Corresponding gene	Expected Product size	Ref.
1-	F:GTGAAATTATCGCCACGTTCGGGCAA	invA gene	284bp	(Oliveira et al.,2003)
	R: TCATCGCACCGTCAAAGGAACC			

DNA samples were amplified in a total of 25 µl as the following: 12.5µl of PCR master mix (promega, USA) , 0.5µl of forward primer, 0,5µl of reverse primer, 6.5µl of PCR grade water and 5 µl of the template. The PCR was performed under the following conditions (primary denaturation: 95˚C / 2 min., secondary denaturation: 94˚C / 1min., annealing: 58˚C / 1 min., extension: 72˚C / 1 min., No. of cycles: 35 and final extension: 72˚C / 10 min. Aliquots of amplified PCR products were electrophoresed in 1% agarose gel. The samples and a 100 bp DNA ladder were loaded in the wells in amount of 5µl of sample. A current of 60 V for 1 hour was passed on the media horizontal electrophoresis unit. Specific amplicons were observed under ultraviolet transillumination compared with the marker. The gel was photographed by a gel documentation system and the data were analysed.

Results and Discussion

In the present study out of 577 studied samples, 60 were *Salmonella* positive. The prevalence of *Salmonella* infection poultry samples were 10.4% and this almost the same percentage reported by (AL-Iedani et al., 2014 and Al-Abadi and Al- Mayah, 2012) that showed the prevalence of *Salmonella* in Basrahprovince were 10.5% ,9.2% respectively. In Mosul, survey on the most important diseases of the digestive system in laying hens and parent stock was took place and Salmonellosis constituted 4% of the cases (Al-NiemaandYoukhana,2012).This variation might be due to the differences in environmental, managemental and geographical distribution or due to the survey did not focused on *Salmonella* infection only and included most important diseases of the digestive system.

All the isolates produced pink red colour colonies against a pinkish background when cultured on BGA and colourless, smooth, pale and transparent raised colonies on MacConkey agar media due to non lactose fermentation properties of *Salmonella*. This result is in agreement with the findings of (Khan et al., 2014; Menghistu et al.,2011; Rumi et al., 2011; Ali et al., 2010 and Ahmed et al.,2008). On XLD media and S.S agar media, all *Salmonella* suspected isolates showed red colonies with black center and white colones with black center respectively. This result is the same that showed by (Abd El Tawwab et al., 2013; Abd El-Ghany et al., 2012; Hasan et al., 2010; Modarressi and Thong, 2010 and Islam et al., 2006).The colonies of *Salmonella* spp. on chromogenic agar were variable in size convex and mauve in color, that previously suggested by (Ali et al., 2016 and Saeed et al., 2013). In Gram's staining, the morphology of the isolated bacteria was small rod shape, Gram negative, single or paired in arrangement which was in agreement with standard morphological characters of the organism as described by (Quinne et al., 2004) also show by (Saha et al., 2012).

Different biochemical tests were used for identification of *Salmonella* organism in this study.

All *Salmonella* were indole, ureas, and oxidase negative, catalase, lysine iron agar and simmone's citrate were positive, triple sugar iron agar (TSI) show positive result - alkaline slant (red), acid butt (yellow) with H_2S and gas production, these results are the same as showed by (Abd El Tawwab et al.,2013 and Quinne et al.,2004). Also, *Salmonella* isolates are unable to ferment lactose, sucrose, although dextrose, maltose, glucose and mannitol were fermented. This result is in agreement with the findings of (Rumi et al., 2011 and Islam et al., 2006). The result of API 20-E showed that 60 isolats were positive to API20-Esystem (Figure 1).

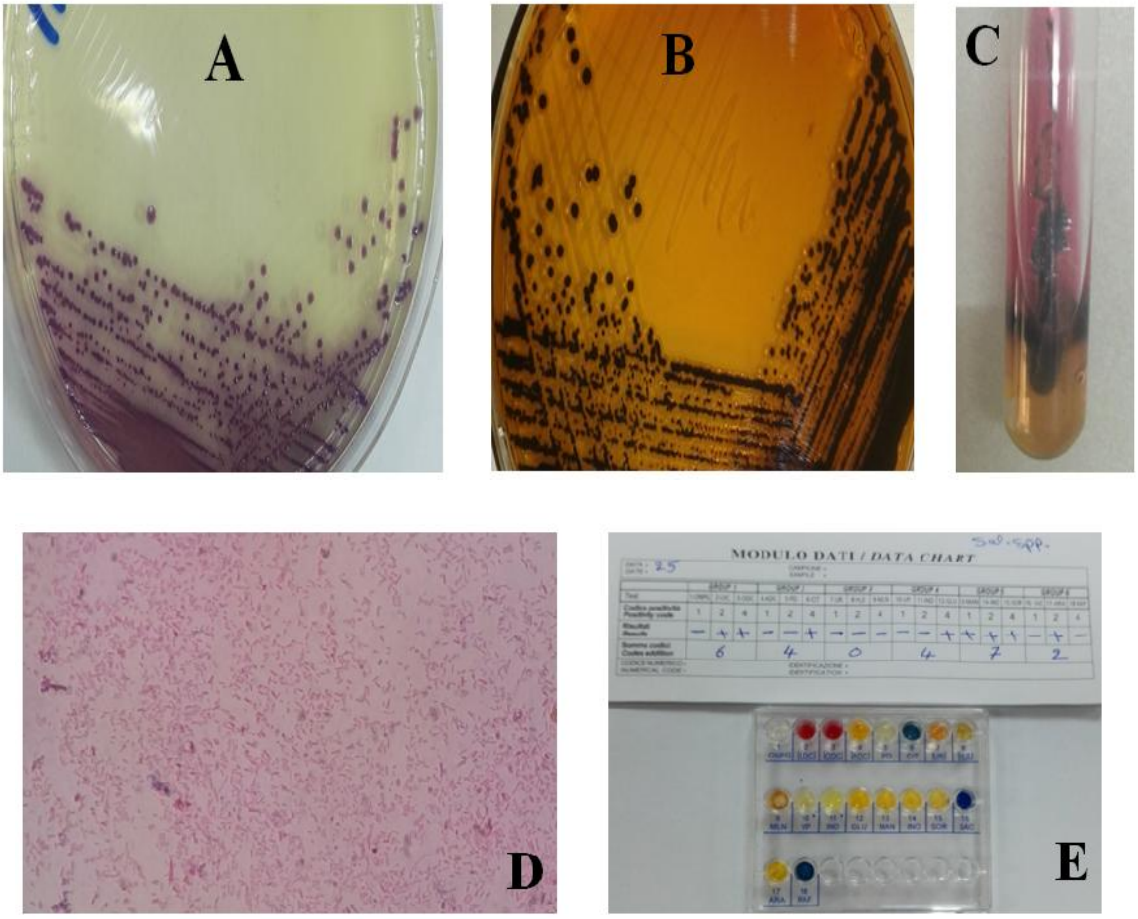

Figure (1) A:chromogenic agar B:Salmonella-Shigella agar C:triple sugar iron agar D:rod, Gram negative Salmonella E:API20-Esystem.

All the *Salmonella* suspected cultures subjected to PCR amplification, generated a product of approximate molecular size 284 bp fragments of *inv*A gene(100%) specific for all members of *Salmonella* species. 100 bp DNA marker (KAPA, South Africa) was used as a molecular weight marker. The band size detected in all the *Salmonella* isolates and analysed by agarose gel electrophoresis(Figure 2).

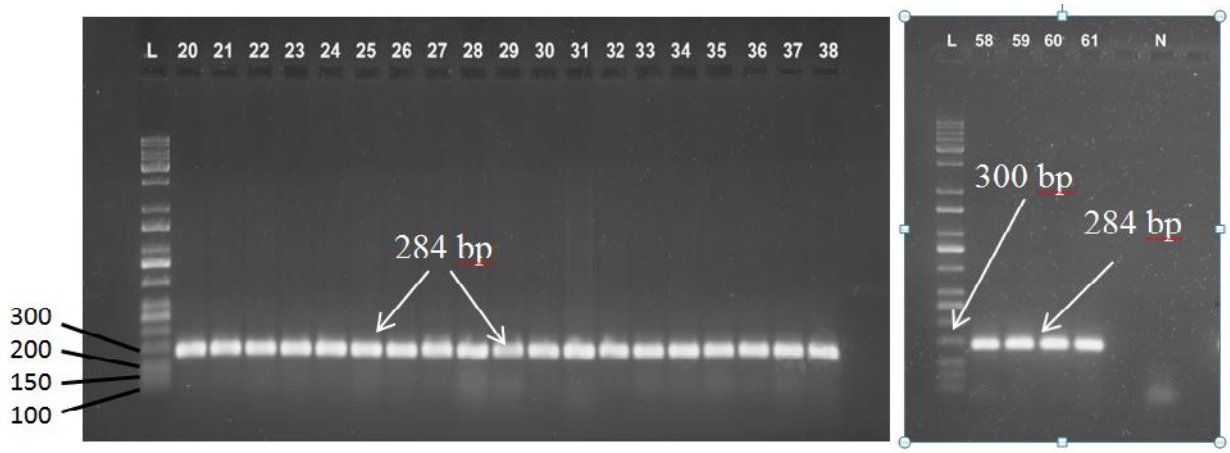

Figure (2) Agarose gel electrophoresis for amplification of *inv*A gene (284bp) of *Salmonella*spp,lane20-38 and 58-61 positive results as *Salmonella* spp. (L): 100 bpMarker . (N):control negative

These results were parallel to those obtained by (Abd El-Ghany et al., 2012 and Oliveira et al., 2003) who reported that *inv*A gene were able to identify all the examined *Salmonella* serovars and routine PCR test in conjunction with traditional identification methods could be effective in providing a more accurate profile of the prevalence of *Salmonella* in poultry flocks. Also this finding agreed with (Abd El Tawwab et al.,2013) who showed, the *inv*A gene was present in all of the isolates (100%) that isolated from chickens and ducks samples, and this gene demonstrated by the presence of a 284 bp PCR amplified fragment.

Due to, this primer sequences are selected from the gene invA, that encodes a protein in the inner membrane of bacteria which is responsible for invasion to the epithelial cells of the host, the ability of *Salmonella* specific primers to detect *Salmonella* species was rapid and accurate (Karmi, 2013 and Shanmugasamy et al., 2011) and now has been recognized as an international standard for detection of *Salmonella* genus (Malorny et al., 2003).

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