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Molecular Detection of *InvA, OmpA* and *Stn* Genes in *Salmonella* Serovars from Broilers in Egypt

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ABSTRACT

The present study was conducted to determinate the prevalence of some virulence genes among *Salmonella* serovars isolated from broiler flocks in Egypt. A total of 55 *Salmonella* isolates were recovered from different samples (liver, yolk sac, gall bladder and caecum) collected from apparently healthy and diseased broilers suffered from whitish diarrhea, dehydration and respiratory distress. Thirty isolates were serotyped into *s*. enteritidis (43.3%) *s*. infantis and *s*. Kentucky (16.6%), s.maloma and s.bardo (6.7%), s.gdansk, s.typhimurium and *s*. blegdame (3.3%). Molecular detections of *invA, ompA* and *stn* virulence genes in 15 *Salmonella* isolates were applied using specific primer sets. The results reported detection of the screening genes in all 15 examined isolates with 100%. In conclusion, these genes appear to have a critical role in pathogenicity of *Salmonella* infection in poultry. So, this help in understanding the process of pathogenicity and design of control and therapy treatment strategies for salmonellosis in broiler chickens.

1. INTRODUCTION

Paratyphoid infection is a serious problem of economic concern with a negative impact to all phases of poultry industry from production to marketing. *Salmonella* spp. Gram negative, motile rods by peritrichous flagella. The genus *Salmonella* is divided into two main categorized species *Salmonella* enterica and *S. bongori*. *Salmonella* enterica is divided into six subspecies, namely S. enterica subspecies enterica, S. enterica subspecies salamae, S. enterica subspecies Arizonae, S. enterica subspecies diarizonae, S. enterica subspecies indica and S.enterica subspecies houtenae **(**Selvaraj et al., 2010).

S. enterica is a highly diverse species that contains over 2600 serovars based on the extensive diversity of the lipopolysaccharide (O) antigens and flagellar protein (H) antigens (Majowicz et al., 2010). Hosts and bacteria have coevolved over millions of years, during which pathogenic bacteria have modified their virulence mechanisms to adapt to host defense systems (Beceiro et al., 2013). Clinical presentation of salmonellosis also depends on many other factors such as the immune status of the host, the serotype of *Salmonella* and the specificity of the interaction of certain serotypes with the host (Jones et al., 2008). Reflecting a complex set of interactions with its host, *Salmonella* spp, harboured multiple genes for virulence expression. Although some of these genes are found on virulence plasmids common to many *Salmonella* serovars, most are encoded within the *Salmonella* pathogenicity islands (SPI) (Heithoff et al., 2008). During evolution, diverse *Salmonella* strains acquired new genetic elements. The majority of virulence factors are encoded on mobile elements and have the ability to transmit via horizontal transfer. New genetic elements have emerged in the pathogenicity of *Salmonella* strains and they play a major role in the clearance of disease (Switt et al., 2012). Therefore, the aim of this study is to detect the prevalence and serotyping of salmonella species in boilers as well as molecular detection of some virulence genes among *Salmonella* serotypes.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 353 samples were collected from broiler flocks at Al-Wady El-Jaded Governorate and subjected to post mortem examinations. The internal organs (liver (n=115), yolk sac (n=59), gall bladder (n=24) and caecum (n=155) were aseptically collected from different ages and subjected to bacterial isolation and identification.

2.2. Bacteriological examination

Isolation of *Salmonella* spp. was done according to ISO 6579 (ISO, 2002): Briefly, 25gm samples were collected in 225 ml buffered peptone water and incubated at 37°C for 24 h, After pre-enrichment 0.1 mL of the broth culture was transferred into 10 mL Rappaport Vassiliadis (RV) broth and incubated at 42°C for 24-48 h. Another 1 mL of the pre-enrichment broth was transferred into a tube containing 10 mL of Muller Kauffman Tetrathionate Novobiocin (MKTTN) broth and incubated at 37°C for 24-48 h. A loopfull from the RV broth and MKTTN was transferred and streaked separately onto the surface of Xylose Lysine Deoxycholate agar (XLD) agar and Hektoen Enteric (HE) agar. Presumptive colonies were selected and purified on nutrient agar plates for further identification.

2.3. Biochemical identification

Identification of the suspected colonies using oxidase reaction, urease test , triple sugar iron agar (TSI), lysine iron (LI), indole test, methyle red (MR) test, voges proskauer (VP) test and citrate test were done according to the standard biochemical procedures described previously (Cruickshank *et al*., 1975).

2.4. Serotyping

Biochemically confirmed *Salmonella* isolates were subjected to serological identification according to Kauffman-White Scheme (Kauffman, 2001) for determination of somatic (O) and flagellar (H) antigens (ISO, 2002).

2.5. Molecular detection of virulence genes

Firstly DNA extraction was done using QIAamp DNA Mini Kit DNA extraction kit (Catalogue no. 51304) according to the manufactures' guidelines, the oligonucleotide primers synthesized by Metabion. The PCR was conducted in a total reaction volume of 25µl consisting of(Emerald Amp GT PCR master mix (2x premix) 12.5 µl ,PCR grade waterr 4.5 µl, PCR grade water 4.5µl, Forward primer (20 pm 1 µl, Reverse primer (20 pmol) 1 µl ,Template DNA 6 µl). The PCR amplification program was as the following table (1).All PCR products were electrophoresed and photographed under UV.

3. RESULTS

3.1. Isolation and identification of *Salmonella* isolates

Isolated *Salmonella* appeared on XLD agar as smooth pink colonies with black center (H2S production) and on HE agar, they appeared as deep blue colour colonies. Application of different biochemical tests revealed the following results; negative oxidase, indole, VP and urea hydrolysis, positive reactions on MR , Citrate , LI (purple color ,H2S production) ,TSI agar(red alkaline slant , yellow acidic butt, with H2S and gas production).

3.2. Occurrence of *Salmonella spp.* in different samples

Out of 353 examined birds, 55 (15.6%) were positive for *Salmonella* isolation**.** The highest prevalence was from liver samples 32.2 %. The most susceptible age was 7-20 days with an incidence of 38.6% (Table2).

Table 1: Primer sequences and cycling conditions of the different primers during PCR

Primer sequencing	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension	Gene and reference
GTGAAATTATCGCCACGTTCGG GCAA TCATCGCACCGTCAAAGGAACC	94˚C 5 min.	94˚C 30 sec.	59˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.	*invA* Rehan, 2004
AGT CGA GCT CAT GAA AAAGAC AGC TAT CGC AGT CAA GCT TTT AAG CCT GCG GCT GAG TTA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 30 sec	35	72˚C 7 min.	*OmpA* Kataria et al., 2013
TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 1 min.	35	72˚C 10 min.	*Stn* Olivera et al., 2003

Table 2. Isolation rate of *Salmonella* isolates from different organs.

Organ	No.of samples	No.of *Salmonella* isolates	The %to total No of samples (353)
Liver	**115**	**37**	**10.5**
Yolk Sacc	**59**	**9**	**2.6**
Gall bladder	**24**	**4**	**1.1**
Caecum	**155**	**5**	**1.4**
Total	**353**	**55**	**15.6**

% was estimated according to the total number of samples (353).

3.3. *Serotyping of Salmonella isolates*

Thirty samples of different isolated the isolated *Salmonella* were identified as eight different serogroups including *S.* Enteritidis, *S.* Infantis, *S.* Kentucky, S.*Maloma* and *S. Bardo*, with the percentage of 43.3 %, 16.6% , 16.6% , 6.7% and 6.7% respectively. Other serotypes including S.Gdansk, *S.Typhimurium* and S. Blegdame were also recorded with the percentages of 3.3 % for each.

3.4. Molecular detection of virulence genes The results revealed that *invA, ompA* and *stn* virulence genes were found in all the 15 examined isolates .They all produced the expected amplification sizes for all the examined genes (Figure1).

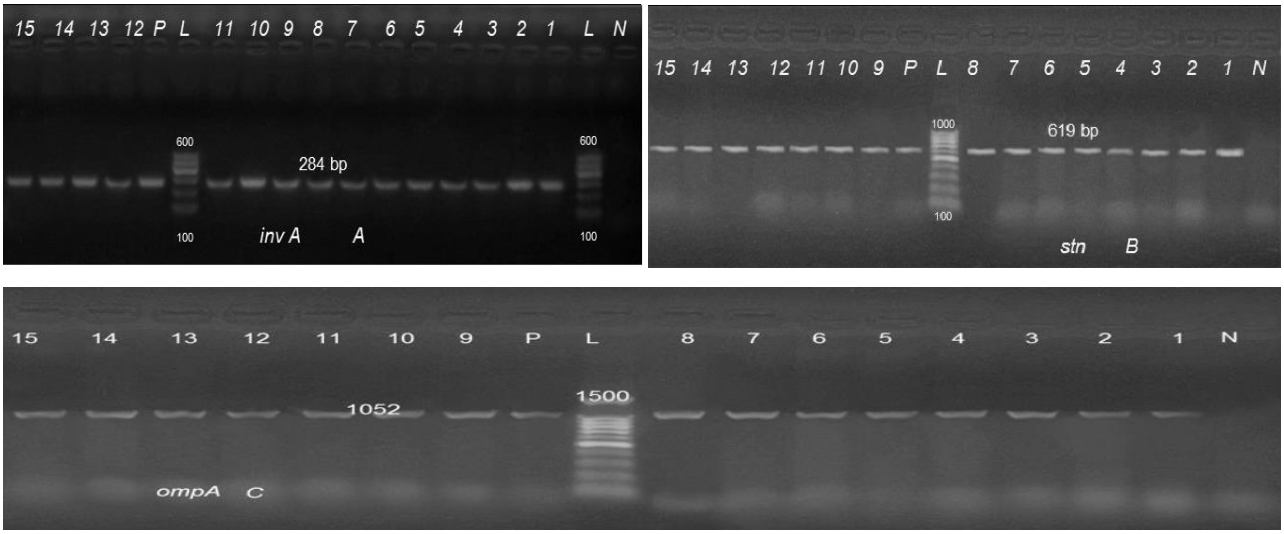

Figure 1: divided into three parts: part I (A) at right above showing amplified product of *invA* gene (284bp) from lane 1-15. Part II (B) at left above showing amplified product of Stn gene (619 bp) and third part III (C) showed amplified product of ompA at (1052 bp) In each photo "P" stands for positive control, "neg": Negative control and numbers indicates lanes with positive and negative samples.

4. DISCUSSION

In this study, 353 samples from different broiler farms at Al-Wady El-Jaded Governorate were examined for the presence of *Salmonella* species. Fifty-five (15.6%) of the examined samples were found positive.

This was nearly similar to other results obtained in (Rehan, 2004 and Mohamed, 2015) where *Salmonella* species were recovered from Dakahlia and Damietta Governorates in Egypt with an isolation rates of 12 % and 12.4%, respectively. Meanwhile, these results were higher than 9.2% and 3.4% reported by (AL-Hakeem 2003 and AL-Abadi and Al-Mayah, 2011) those obtained in Basrah and Musol province in Iraq respectively. These differences in the overall prevalence of *Salmonella* may be related to several factors such as environment, hygienic conditions of the farm and health status of the examined chickens. The prevalence of the isolated bacteria from different internal organs of the examined broilers in this study showed that the highest percentage of *Salmonella* was recorded in liver (32.2%) followed by gall bladder (16.7%), yolk sac (15.3%) and caecum (3.2%). These results are in contrast with (Dhahar *et al*., 2011 and AL-Iedani et al., 2014). On the other hand, (AL-Abadi and Al-Mayah ,2010) recorded that the highest percentage of *Salmonella* isolation was from cecum which is the primary sites of colonization of *Salmonella* due to the anatomical and structural location that allows the cecum to act as a blind sac with low content flow rate.

Salmonella Enteritidis prevalence with higher incidence in this study is correlated to (Dawod, 2012; Ibrahim, 2014 and Mohamed, 2017). Who detected *Salmonella* Enteritidis as higher prevalence. However *Salmonella Typhimurium*, Infantis, Kottbus, Kentuky, Minnesota were detected as higher prevalence by (Voss Rech et al., 2015 Muammer et al., 2016 and Abde-Rahim, 2016) respectively. It has been reported that *invA* gene is present only in *Salmonella* species and therefore is used as a golden marker in genetic diagnosis of *Salmonella* species as previously described (O'Regan et al., 2008). In our study the *invA* gene was detected with 100% in all examined *Salmonella* serovars, high prevalence rates of *invA* virulence gene in *Salmonella* serovars has also been reported by other workers (Karmi, 2013 and Chaudhary et al., 2015). In addition, the result of our study revealed that the *stn* gene was present in all of the isolates 100%. (Huehn et al., 2010) revealed that the *Salmonella* enterotoxin *stn* gene encodes *Stn* protein, causing gastroenteritis with symptoms that include nausea, vomiting, abdominal pain, fever, and diarrhoea. In accordance, (Murugkar et al. 2003 and Shalaby, 2012) reported that *stn* gene was detected in all the isolated *Salmonella* strains. Moreover, (Zou et al., 2012) identified *stn* gene in all 425 isolates (100%) of poultry origin. Regarding to *ompA* gene, this gene have a significant role in adaptation of *Salmonella* to environmental stresses, as well as adhesion, invasion and damage of host tissue or evasion of host defense resulting in clinical disease or death (Krishnan and Prasadarao,2012). In this work it was clear that *ompA* gene was detected in all isolates.

These agreements with results of (Kataria et al., 2013) reported that the *ompA* gene is found specifically in all 68 tested *Salmonella* serovars by PCR and (Okamura et al., 2012) who confirmed that *ompA* is well conserved among the different *Salmonella* serovars.

Conclusion

Our results revealed that different salmonella serotypes was obtained from broilers as *s*. enteritidis (43.3%) *s*. infantis and s. Kentucky (16.6%), s.maloma and s.bardo (6.7%) , s.gdansk , s.typhimurium and *s*. blegdame (3.3%). Also PCR method can use as an important technique in the diagnosis of virulence genes (*invA, stn and ompA*) of *Salmonella* serotypes. In addition to the importance of invA gene that could be used as marker for rapid and accurate detection of *Salmonella* species, Furthermore, the *stn and ompA* genes contained sequence unique to *Salmonella* strains, this making this gene a suitable target for detection of *Salmonella* strains in field samples. In conclusion this work provides a screening data for some of the most important *Salmonella* virulence genes in Egypt.

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