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A study of outer membrane protein (OMPs) genes for detection of salmonella organisms in poultry farms

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A B S T R A C T

Bacteriological examination of internal organs obtained from diseased layers, broilers and baby chicks revealed isolation of salmonellae with an incidence 5%, 2.5% and 7.1%, respectively. Eleven Salmonella isolates were recovered out of 250 poultry samples. The highest percentage rate was in liver 4%, followed by intestine 2.4% and finally spleen 0.8%. Salmonella organisms were recovered from ovary of layers with an incidence 3.33%. Serological identification of Salmonella isolates revealed that the most prevalent serovars was. Enteritidis (3) followed by. Gallinarum (2) and Rissen (2) then *S.* Kentukey, *S.* Florida, *S.* Lomita and *S.* Sontheim (one isolate for each). The goal of this study was to evaluate the suitability of the outer membrane genes *Omp*A and *Omp*F for detection of Genus *Salmonella* in clinical samples. *Omp*A gene was detected in 100% of the examined clinical samples while *Omp*F gene was detected in 14 clinical samples. Three samples were positive by *Omp*F.PCR more than cultural method. It was concluded that PCR protocol decrease the time needed for the detection of Salmonella *Omp*A gene couldn't discriminate genus *Salmonella* from other non-Salmonella organisms in clinical samples .AS it amplified *Omp*A in other *Enterobacteriaceae*. However, *Omp*F gene is promising tool for detection of Genus *Salmonella*.

Key words: *S. Enteritidis, outer* membrane protein genes, PCR

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1. INTRODUCTION

Salmonella infections in chicken continue to be a major problem worldwide. Substantial economic losses were manifested through mortality and poor growth of infected chicken as well as the hazard of causing food poisoning to humans (Painter et al., 2013). Avian salmonellosis is an inclusive term designated a large number of acute and chronic diseases of poultry caused by member of genus *Salmonella*. Salmonellosis in chicken can be classified into three diseases: pullorum disease caused by *Salmonella* Pullorum, fowl typhoid caused by *S.* Gallinarum and paratyphoid infection due to diverse group of serovars related to food born illness in human (Davies., 2013). The polymerase chain reaction (PCR) represents a major advanced terms of the speed, sensitivity and specialicity of diagnostic methods, and has been increasingly used to identify several bacterial species from food and clinical samples (Morales., 2014). Salmonella OMPs play an important role in the virulence and immunological properties of bacteria, *Omp*A and *Omp*F are the most abundant genes in Salmonella outer membrane protein. The main role of *Omp*A is to provide integrity to the membrane by ensuring physical linkages between the outer membrane and the underlying peptidoglycan layer. It also serves as receptor to some of bacteriophages and the colicins (Kataria et al.,2013) Salmonella *Omp*A is immunostimulatory as

demonstrated by stimulation of *IFN*-α production, enhance expression of major histocompatibility complex and co stimulatory molecules in dendritic cells and/or Tcells (Lee et al., 2010). *OmpF* play a role in the physiology of the bacterium by allowing the small hydrophilic molecules to pass through the channel. (Shaw and Riederer., 2006) and transport of nutrients and other molecules (Koebnik et al., 2000). The objective of the present work was to estimate the prevalence of Genus *Salmonella* in poultry, provide an update recognition of Salmonella serovar and detection of Salmonella organisms from clinical samples using *Omp*A and *Omp*F genes by PCR.

2. MATERIAL AND METHODS

2.1. Sampling

A total number of 250 diseased chicken of different ages were checked for the presence of salmonellae in different flocks (layers n=60, broiler n=120 and baby chicks n=70). The birds were obtained from farms at El-Dakahlia governorate farms, Egypt. Samples were collected from internal organs liver, intestine, spleen in addition to 60 ovaries samples from layers. Bacteriological examination according to (ISO6579:2002).Apiece of twenty five gram of each sample was transferred to 225 ml of buffered peptone water (BPW)and incubated for 18hr at 37 °c. 1ml of the pre-enrichment was transferred to 10ml of muller-kauffmnn tetrathionate novobiocin broth (MKTTn) and incubated at 37 °c for 24 hr., another 0.1ml of the pre-enrichment was transferred to 10 ml of Rappaport-vasiliadis soya broth (RVS broth) and incubated at 41.5 °c for 24hr .Each enrichment culture was streaked onto Xylose Lysine Deoxycholate (XLD) and Salmonella -Shigella agar (S.S agar) incubated at 37 °c for 24hr . The suspected colonies were purified and identified by API20E according to manufacture instructions

2.2. Serological identification:

According to White Kauffman- Leminor scheme described by Grimont and Weill (2007) using SIFIN antisera Berliner Allee 417/321, D-13088.

2.3. *Detection of Genus Salmonella by outer membrane genes OmpA and OmpF PCR***.**

Primers Source: Biobasic (Canada). Table (1). Extraction of DNA: A total of 20 clinical samples,11 salmonella positive samples and 9 suspected samples obtained from birds with symptoms of salmonellosis and yield negative result by cultural method. DNA extraction was done according to QIAamp DNA mini kit instructions.

Preparation of PCR master mix for PCR according to Emerald Amp GT PCR mastermix (Takara) code no. RR310A kit.

Cycling condition: one cycle primary denaturation at94˚C

for 5 min. then secondary denaturation at94˚C for 30 sec; OmpA annealing at55˚C for1 min and at50˚C for45 sec for OmpF; Extension at 72˚C for1 min for OmpA and 45 secs for OmpF and final extension at72˚C for 12 min for OmpA and 10 min for OmpF.

Amplified PCR products was electrophoresed in 1.5 % agar by Agarose gel electrophoreses according to Sambrook et al., (1989) and visualized by u.v transilluminator.

3. RESULTS

Bacteriological examination of internal organs of poultry revealed the isolation of Salmonella from diseased birds with overall incidence 4.4%. Salmonella was recovered from layers, broilers and baby chicks with an incidence 5%, 2.5% and 7.1% respectively as shown in (Table 2)

3.1. Prevalence of Salmonella:

Salmonellae were isolated from liver, intestine, spleen, and ovaries with incidence of 4%, 2.4%, 0.8% and 3.3% respectively. It was noticed that the highest percentage of

recovery was (7.1%) from liver of baby chicks followed by intestine of baby chicks (4.28%). (Table 3)

3.2. Serological identification:

Serological identification of salmonellae recovered from different organs revealed that isolation of *S. Enteritidis*, *S. Rissen* and *S. Kentuky* from layers, *S.Enteritidis*, , *S.Lomita* and *S.Gallinarum* from broilers and *S.Rissen*, and *S. Sontheim*, *S.Enteritidis, S.Gallinarum* and *S.Florida* from baby chicks.(Table 4)

3.3. Detection of Genus Salmonella using OmpA & OmpF genes by PCR:

The used primer of *Omp*A gene multiplies a region 1052 bp in all examined clinical samples (100%) fig (1). Detection of *Omp*F gene: 14 clinical samples harbour *Omp*F gene. It was noticed that 3 samples were positive more than cultural method (no 15, 16,19) as shown in fig (2).

Table (1): Oligonucleotide primers sequences

Target gene	Primers sequences	Amplified segment (bp)	Reference
*Omp*A	AGT CGA GCT CAT GAA AAA GAC AGC TAT CGC AGT CAA GCT TTT AAG CCT GCG GCT GAG TTA	1052	Kataria *et al*., 2013
*Omp*F	CCT GGC AGC GGT GAT CC TGG TGT AAC CTA CGC CAT C	519	Tatavarthy and Cannons, 2010

Table (2) Prevalence of salmonellae recovered from layers, broilers and baby chicks:

Type of flocks	no of Examined birds	No of samples	No of samples +ve	%
			NO	% *
1-Layers	60	240	3	5
2-Broilers	120	360	3	2.5
3- Baby chicks	70	210	5	7.1
4-Total	250	810	11	4.4 **

* The percentage is calculated according to the No. of each type of chicken. ** The percentage is calculated according to the total No. of chicken.

Table (3) Prevalence of Salmonella recovered from different organs of diseased chickens

Number& type of samples	Number of positive examined samples	% of positive*	Layers (60)		Broiler (120)		Baby chicks (70)	
				%**		%**		%**
Liver (no, 250)	10	4%	2	3.3	3	2.5	5	7.1
Intestine (no, 250)	6	2.4%	2	3.3	1	0.83	3	4.28
Spleen (no, 250)	2	0.8%	-	0	1	0.83	1	1.4
Ovary (no, 60)	2	3.33%	2	3.3	-	0	-	0

* The percentage is calculated according to the total number of each organ. ** The percentage is calculated according to the number of samples examined from each type of chicken flock.

Table (4) Serological identification of salmonellae recovered from the internal organs of different chicken flocks

Poultry	Organ	Strain
Layers	Intestine and ovary	*S*.Enteritidis
	Intestine and liver	*S*.Rissen
	Liver and ovary	*S*.Kentuky
Broilers	Liver and intestine	*S*.Enteritidis
	Liver	*S*.Lomita
	Liver and spleen	*S*.Gallinarum
Baby chicks	Liver and intestine	*S*.Rissen
	Liver and spleen	*S*.Sontheim
	Intestine and liver	*S*.Enteritidis
	Intestine and liver	*S*.Gallinarum
	Liver	*S*.Florida

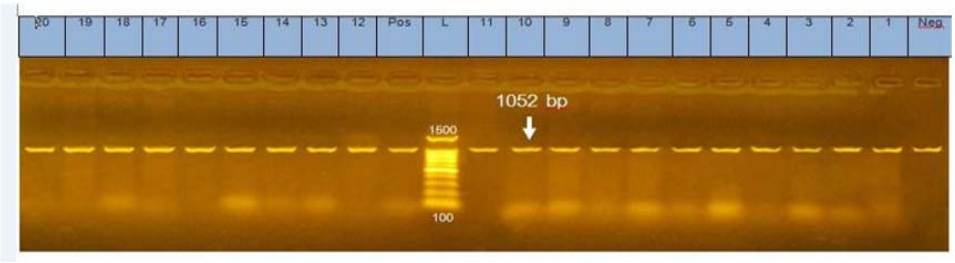

Fig (1) Agar gel electrophoresis showing PCR with amplification of 1052 bp fragment for *Omp*A gene. *Marker*: (cat. no. 239035) supplied from QIAGEN (USA). Number of bands: 6. Size range: 100-600 bp. Lane: from (1) to (20) +ve at 1052 bp. Control positive sample S.Typhimurium stander strain (NTCTC 12023/ATCC ®14028). Control negative sample *Echerichia coli*(NCTC 12923/ ATCC ® 8739).

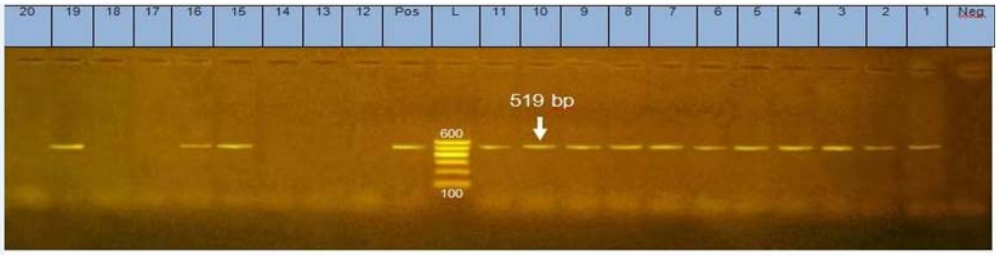

Fig (2) Agar gel electrophoresis showing PCR with amplification of 519 bp fragment for *Omp*F gene. *Marker*: (cat. no. 239045) supplied from QIAGEN (USA). Number of bands: 11
Size range: 100-1500 bp. Lane: from (1) to (13) and (15,16 and 19) +ve at 519 bp. (12,13,14,17,18,20) –ve at 519 bp. Control positive sample S.Typhimurium stander strain (NTCTC 12023/ATCC ®14028). Control negative sample *Echerichia coli* (NCTC 12923/ ATCC ® 8739).

4. DISCUSSION

Salmonella organisms is a leading cause of foodborne illness in many countries which poultry being important vehicle of transmission (Threlfall et al., 2014). In the present study 11 isolates recovered from internal organs of 250 birds had symptoms of salmonellosis with an overall incidence 4.4% The percentage of recovery was 5%,2.5% and 7.1% from layers, broilers and baby chicks respectively (Table 2).These results were agreed to large extent with Balala et al. (2006) who recovered 16

Salmonella isolate out of 325 samples with incidence (4.9%) , Liu et al., (2010) who reported that salmonellae could be isolated with a percentage rate 4.5% from five chicken farms in Shenghi, and Abd El-Ghany et al. (2012) who isolated Salmonella in range of 3.84% to 5.06% from four chicken flocks in Kalubia governorate, Egypt . In most cases salmonellae isolates were recovered from more than one organ among the examined chickens .The highest percentage of recovery was liver followed by ovaries then intestine and finally spleen 4%,3.33%,2.4% and 0.8% respectively (Table 3).Salmonella were recovered from liver samples of diseased poultry with incidence of 5.4% by Sharawy (2006) and 2% by Abd El Fatah (2014) .On the other hand Akond et al. (2012)found that the highest proportion of Salmonella contamination was in the intestinal fluid samples 60%.

Poultry were commonly infected with a wide variety of *Salmonella* serovars. One serovars may be a predominant isolate in a country for several years before it is replaced by another serovar according to Bacci et al., (2006) The most prevalent serovar was *S.Enteritidis* which isolated from layers, broilers and baby chicks .This result coincide with Bacci et al., (2006), El Ebeedy (2011) and Abd El Fatah (2014) who reported that S.*Enteritidis* is predominant among poultry isolates. The emergence of *S.Enteritidis* as the leading cause of human Salmonellosis in many countries was attributed to this serotypes unusual ability to colonize the ovarian tissue of hens and be present within the contents of intact shell eggs (Threlfall et al., 2014). *S. gallinarum* was recovered from broilers and baby chicks. *S. gallinarum* is an important chicken pathogen and seldom causes disease in human. In Egypt, Zoo El Fakar and Rabie 2009) recovered *S.* Gallinarum with an incidence 2.2% and Abd El Fatah (2014) recovered *S.*Gallinarum from intestine and oviduct of layers Culture technique was universally recognized as standard method for the detection of bacterial pathogens such as Salmonella (White et al., 2002).These techniques generally take long time and were less sensitive compared to PCR based method (Malorny et al., 2003). Several studies have successfully targeted *inv*A gene for the detection of Salmonella species by PCR. However, as *inv*A gene is a virulence gene located on the Salmonella pathogenicity island 1 (SPI 1) acquired by horizontal gene transfer, it may be genetically unstable or absent in some serotypes including Salmonella serotype Senftenberg (Tatavarthy et al., 2010). A total of 20 clinical samples (internal organs) were examined for the presence of outer membrane protein genes *Omp*A and *Omp*F. Eleven culture positive samples and 9 suspected samples. *Omp*A gene was detected in 100% of the examined clinical samples this gene is conserved among *Salmonella* serovars and the conserved nature of this gene indifferent Salmonella serovars may be used for detection of Salmonella in food or clinical samples Kataria et al., (2013). In the present study it was noticed that *Omp*A could be amplified a region of 1052bp in all clinical samples even in negative control which had *E. coli* this finding is in accordance with the finding of Zhang et al., (2009) who reported that, *Omp*A gene could be detected in *E. coli, K. pneumonia, and S.*flexneri isolates. And other Gram- negative bacteria. *Omp*F gene was detected in 14 clinical samples, 11 culture positive samples and 3 samples were positive by *Omp*F PCR more than culture method. This result agrees to large extend with the negative result by culture might be explained by loss of viability of bacteria with specimen handling or lack of sensitivity of culture method**.** Tatavarthy and Cannons (2010) who found that *Omp*F was detected in 218 Salmonella strain tested .It was present in all the six Salmonella subspecies demonstrating 100% inclusivity while *Omp*F was absent in 180 non salmonella strains (22 genera)examined indicating 100% exclusivity .*Omp*F gene couldn't be detected in the most common

foodborne pathogens tested including *E. coli, Shigella* spp., *Staphylococcus* spp. *Listeria* spp., *Campylobacter* spp. *and Vibrio* spp. (Armand-Lefevre et al. 2003). It was clear that uses of *Omp*F gene is a promising tool for detection of salmonellae as it could discriminate genus *Salmonella* from other non Salmonella organisms in clinical samples.

5. REFERENCES

Abd El-Ghany, W.A., Soumaya, S.A., El-Shafii Hatem, M.E. 2012**.** A survey on Salmonella species isolated from chicken flocks in Egypt. Asian Journal of Animal and Veterinary Advances,7: 489-501**.**

Abd Elfatah, A.A. 2014**.** Detection of multidrug resistant Salmonellae. M.V.Sc thesis, Fac. of Vet. Med. Cairo univ.

Akond, M.A., Shirin, M., Alam, S., Hassan, S.M.R., Rahman, M.M., Hoq, M. 2012**.** Frequency of drug resistant *Salmonella* spp. isolated from poultry samples in Bangladesh. Stamford Journal of Microbiology 2(1): 15-19.

Armand-Lefevre, L., Leflon-Guibout, V., Bredin, J., Barguellil, F., Amor, A., Pages, J.M., Nicolas-Chanoine, M.H. 2003. Imipenem resistance in Salmonella enterica serovar Wien related to porin loss and CMY-4 beta-lactamase pro-duction. Antimicrob Agents Chemother 47: 1165–1168.

Bacci, D., Bonardis, S., Ferronic. 2006. Poultry as Salmonella infection source for workers in laying hens farms and egg paking.Vet Res,comm., 30: 93-97.

Balala, L.M., Rovira, H.G., Vizmanos, M.F.C., Bernardo, F.A.E., Divina, B.P. 2006. Isolation, Serologic Identification and Antibiotic Sensitivity Testing of *Salmonella* spp. in Chickens. Philipp. J. Vet. Med., 43(2): 63-70.

Davies, R.H., Wales, A.D. 2013. Salmonella contamination of cereal ingredients for animal feeds. Vet Microbial. 25; 166(3-4): 543-9.

El-Ebeedy, M.A.L. 2011. virulence factors in Salmonella of human origin Thesis of fac. Of Pharmacy in Benisuif university

Grimont Patrick, A.D., Weill, F.X. 2007. Antigenic Formulae of the Salmonella serovars: Who Collaborating Centre for Reference and Research on Salmonella. 2007 9th edition.

Kataria, J.L., Kumar, A., Rajagunalan, S., Jonathan, L., Agarwal, R.K. 2013. Detection of OmpA gene by PCR for specific detection of Salmonella serovars. veterinaryworld.org/6/16

Koebnik, R., Locher, K.P., Van Gelder, P. 2000 Structure and function of bacterial outer membrane proteins: barrels in a nutshell. Mol.Microbiol., 37: 239-253.

Lee, J.S., Jung, I.D., Lee, C.M., Park, J.W., Chun, S.H., Jeong, S.K. et al. 2010. Outer membrane protein a of Salmonella enterica serovar Typhimurium activates dendritic cells and enhancesTh1 polarization. BMC Microbiol. 10: 263.

Liu, W.B., Chen, J., Huang, Y.Y., Liu, B., ShiX, M. 2010. Serotype, genotype, and antimicrobial susceptibility profiles of Salmonella from chicken farms in Shanghai. J. Food Prot. 73(3): 562-7.

Malorny, B., Hoorfar, J., Bunge, C., Helmuth, R. 2003. Multicenter validation of the analytical accuracy of Salmonella PCR: towards an international standared. Appl. Environ. Microbiol., 69(1): 290-296

Morales, C.A.R., Gast, Guard-Bouldin, J. 2014. Kataria, J.L., Kumar, A., Rajagunalan, S., Jonathan, L., Agarwal, R.K. 2013. Detection of *OmpA* gene by PCR for specific detection of *Salmonella* serovars.age of avian and reproductive tract

tropism with sequence divergence adjacent to the 5S ribosomal subunit rrfH of Salmonella enterica. FEMS Microbiology Letters 264: 48–58.

Sambrook, J., Fritscgh, E.F., Mentiates, 1989. Molecular cloning. A laboratory manual. Vol 1, Cold spring Harbor Laboratotry press, New York.

Sharawy, H.I. 2006. new approaches for studying different aspects of Salmonellosis. Ph.D. in microbiology cairo university.

Shaw, M.M., Riederer, B.M. 2006. Sample preparation for two-dimensional electrophoresis. Current Advancements in the Methodology. *G.I.T Laboratory Journal*. 6: 302-303.

Tatavarthy, A., Cannons, A. 2010. Real-time PCR detection of Salmonella species using a novel target: the outer membrane porin F gene (*Omp*F). Lett. Appl. Microbiol. 50: 645-652.

Threlfall, E.J.J., Wain, T., Peters C., Lane, E., De Pinna, C.L.,

Little, A.D. Wales, R.H., Davies. 2014. Egg-borne infections of humans with salmonella: not only an S.enteritidis problem. World's Poultry Science Journal 70 (01):15-26.

Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R. Angulo, F.J., Griffin, P.M. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerging Infectious Diseases 19: 407–415.

White, P., McGillK Collins, J.D., Gormely, E. 2002.The prevalence and PCR detection of Salmonella contamination in raw poultry Vet.Microbiol., 89: 53-60.

Zhang, X.L., Jeza, V.T., Pan, Q. 2009. Salmonella Typhi: from a human pathogen to a vaccine vector. Cell Mol Immunol;5(2): 9-15

Zoo El-fakar Saher, A., Rabie Nagw, S. 2009. Immunogenic properties of outer membrane protein of Salmonella in chicken Global Veterinaria 3(2): 75-79.