JWPR
Journal of World's Poultry Research

J. World's Poult. Res. 3(2): 63-67, 2013

Original Article

Isolation and Molecular Identification of Salmonella *typhimurium* from Chicken Meat in Iraq

Aseel A. Saeed[1], Mayada F. Hasoon[2] and Majed H. Mohammed[3, 4*]

[1] *College of Veterinary Medicine, University of Qadisiyah, Iraq*
[2] *Scientific Research Centre, Faculty of Science, Duhok University, Iraq*
[3] *College of Veterinary Medicine, Iraq, Baghdad, University, Iraq*
[4] *Faculty of Veterinary Medicine, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia*

*Corresponding author's email: majed_mohammed@putra.upm.edu.my

ABSTRACT

This study was conducted to determine the prevalence of Salmonellae contamination of chicken meat imported from different origin to local markets in south of Iraq (Diwaniya). The bacteria were cultured, isolated and biochemically characterized by the analytical profiling index (API 20E system). The *16s rRNA and invA* gene primers were selected specifically for the detection of *Salmonella* to amplify a 406 and 558 bp DNA fragments, respectively. The results of this study showed that 22 *Salmonella* isolates were detected by polymerase chain reaction (PCR) from 100 chicken meats and only 7 isolates out of 22 were identified as *S. typhimurium*, the highest percent of isolates were 83.8 % for India origin and the lowest percent were 25% from Jordan origin.

Keywords: Chicken meat, Salmonella *typhimurium*, PCR

INTRODUCTION

Food borne diseases are main problems, particularly in developing countries and cause the majority of illnesses and death around the world. Food is the most important vehicle that transmits the microorganisms to human (Varnam, 1991), among these microorganisms Salmonellae still a major cause of food-borne human disease in most parts of the world (Soultose et al., 2003; Carraminana et al., 2004). Poultry and poultry products are frequently contaminated with Salmonellae that can be transmitted to humans through the handling of raw poultry carcasses and products, or through consumption of undercooked poultry meat (Bailey and Cosby, 2003; Kimura et al., 2004).

Poultry meat is contaminated with Salmonellae not only by infected poultry, but also by cross-contamination with faeces, water, instruments and worker's hands during the slaughter process and handling. Chicken might thus provide the main transmission route of infection, especially with the increasing consumer demand for this food. This study was undertaken as a prelude to exposure assessment to determine Salmonellae spp. contamination associated with chicken meats was imported from different sources in the markets of south Iraq (Diwaniya).

MATERIALS AND METHODS

Sample collection

Chicken samples were collected from different market in al-diwania city with different origin include different trademark (al-kafeel , al murad , thighs U.S.A, Turkish Chicken, Chicken JD) about 25 g of meat sample were placed in enrichment medium tetrathionate broth and then transported to microbiology laboratory at the College of Veterinary Medicine / Diwaniya University, for 18-24 hr at 37°C.. This study was occurred during the period from December 2011 and carry on June 2012.

Isolation and identification of Salmonella spp.

The samples were cultivated on selective media such as bismuth sulphate agar, chromogenic agar and incubate at 37C° for 18-24 hr. Samples were subjected to biochemical tests such as (TSI), Sulfide-Indole-(SIM), (MRVP), Urea, and Api20-e system.

Specific Primers Sequence Used for PCR Amplification

The primers used for the detection specific sequence of *16s rRNA* gene ribosomal genes of *Salmonella* spp (White et al., 2002). And *invA* gene encoding proteins of a type (T3SS) III secretion system (Baay et al 1993).These primers are specific for designed in this study by using NCBI Gene Bank and Primer: online and provided by (Bioneer company, Korea) as following Table 1.

To cite this paper: Saeed A.A., Hasoon M.F. and Mohammed M.H. 2013. Isolation and molecular identification of Salmonella *typhimurium* from chicken meat in Iraq. *J. World's Poult. Res.* 3(2): 63-66.
Journal homepage: http://jwpr.science-line.com/

Table 1. Specific primers used for the detection specific sequence of *16s rRNA gene* and *invA gene*

Sequences	Orientation	Position	Size of PCR product(bp)
CGG.,ACG,GGT,GAG,TAA,TGT,CT	Forward	*16s rRNA*	406
GTT,AGC,CGG,TGC,TTC,TTC,.TG	Reverse		
ATG,CCC,GGT,AAA,CAG.ATG,ATG,AG	Forward	*invA*	558
CTC,GCC,TTT,GTC,GGT,TTT,AG	Reverse		

DNA extraction

The bacterial DNA was extracted by using Genomic DNA kit according to the manufacturer's instruction (USA).

DNA Amplification:

The amplified DNA products from *Salmonella spp*. specific-PCR were analyzed with electrophoresis on 1% agarose gel stained with ethidium bromide and visualized by UV illumination depending on DNA marker (2000 bp DNA ladder).

Preparation master mix for Detection of *16s rRNA* and *invA* genes

For the detection of *Salmonella* spp. and *S. typhimurium* by PCR. The PCR amplification mixture (20µl) which was used for the detection each gene includes 5 µl of (PCR PreMix Lyophilized**)**, which provided by Bioneer (Korea.) include: bacterially derived Taq DNA polymerase; dNTPs which include: 400 µM of each dATP, dGTP, dCTP, dTTP; 3mM of $Mgcl_2$. Yellow and blue dyes as loading dye, 5 µl of template DNA, 1.5 µl of each forwarded and reversed primers and 7. µl pcr water to complete the amplification mixture to 20 µl. The PCR tubes containing an amplification mixture were transferred to thermocycler and started the program for amplication of the *16s rRNA* and *invA genes* . 30 cycles of PCR, with 1initial denteration 1 cycle 95C˚ for I min then 5 min at 95C˚ (denaturation), 30 s at 55 C˚ (annealing), and 45s at 72 C˚ (extension), and 1 cycle for 7 min at 72 C˚.

RESULTS

Culture methods

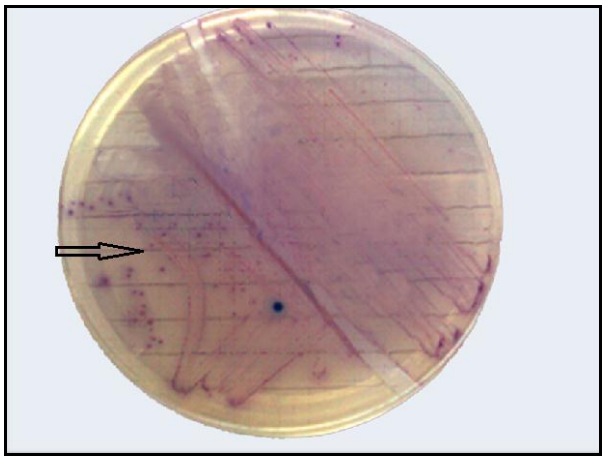
The total percentage of isolation on tetrathionate broth, bismuth sulphate agar, chromogenic agar was 55% (55/100), 60 % (33/55), 87.8% (33/29) respectively, the highest percent of isolation was India origin. The colonies of *salmonella spp.*on chromogenic agar were variable in size convex and mauve in color.

Confirmatory isolation of *salmonella spp*. and *S.typhimurium* by using Api20-E

Salmonella isolates were showed positive productive results to H_2S, TSI, SIM and gives negative for indole, vo-gs Proskauer and ureas. The total percentages of these tests were 89.6% (29 \ 26).While the result of API 20-E showed that 25 isolated positive to API20-Esystem from 26 with percentage 96.1% (Table 3).

Single plex PCR

The total percentage was 92 % (23/25) for chicken meat and the higher percent for isolation salmonella spp. by *16s rRNA* gene were al-kafeel and U.S.A thighs 100% while the lower percent was Turkish origin 75%**.** The total percentage for detect *invA* gene for *S.typyimuirim* serotype was 30.4 % (7/23). And the highest percent of isolation of *S.typyimuirim* was 50% from India origin while the lower was 0 % from Turkish origin. (Table 4), (Figure 2 and 3).



Figeur 1. The Results of isolation *Salmonella spp*. using cultural methods. Colonies of *salmonella spp*. on chromogenic salmonella agar (The arrow shows variable size and mauve in color).

To cite this paper: Saeed A.A., Hasoon M.F. and Mohammed M.H. 2013. Isolation and molecular identification of Salmonella *typhimurium* from chicken meat in Iraq. *J. World's Poult. Res.* 3(2): 63-66.
Journal homepage: http://jwpr.science-line.com/

Table 2. Results of *salmonella spp*. Isolation by using culture methods from chicken meat sample.

Culture mdia Samssample origin		Tetrathionate broth			Bismuth sulphate agar			Chromogenic agar		
Origin		**No. of tested sample**	**No. of positive**	**%**	**No. of tested sample**	**No. of positive**	**%**	**No. of tested sample**	**No. of positive**	**%**
Jordan	chicken JD	20	12	60	12	7	58.3	7	6	85.7
Turkish	casken oglo	20	9	45	9	5	55.5	5	5	100
Brazil	al-kafeel	20	10	50	10	6	60	6	5	83.3
India	al-murad	20	11	55	11	7	63.6	7	7	100
U.S.A.	thighs	20	13	65	13	8	61.5	8	6	75
Total		100	55	55	55	33	60	33	29	87.8

Table 3. Results of Biochemical test and API20-E system

Test Sample origin		Biochemical test			API20-E system		
Origin	**trademark**	**No. of tested sample**	**No. of positive**	**(%)**	**No .of tested sample**	**No. of positive**	**(%)**
Jordan	chicken JD	6	5	83.8	5	5	100
Turkish	casken oglo	5	4	80	4	4	100
Brazil	al-kafeel	6	6	83.3	6	5	80
India	al-murad	6	5	83.8	5	6	100
U.S.A.	thighs	6	6	100	6	6	100
Total		29	26	89.6	26	25	96.1

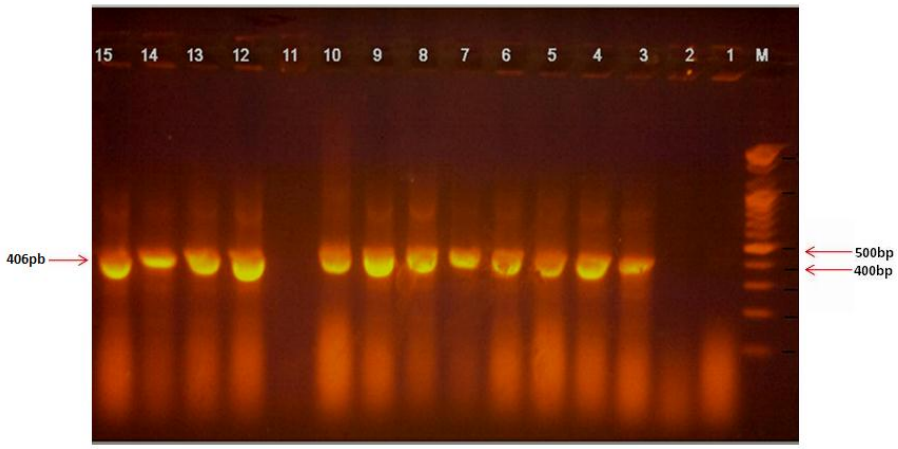


Figure 2. DNA amplification of a 406 bp of salmonella spp. detecting *16s r RNA* gene using singleplex PCR lane 1 control, lane 2,11 negative results ,lane 3,4,5,6,7,8,9,10,12,13,14,15 positive results as *salmonella spp*. Lane M 2000bp marker (ladder).

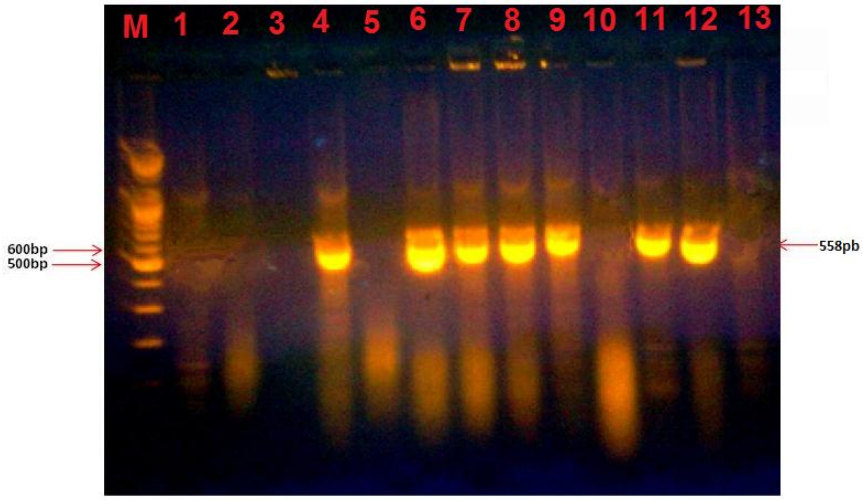


Figure 3. DNA amplification of a 558 bp of *Salmonella spp* .detecting *invA* gene using singleplex PCR lane 1 conterol results ,lane,4, 6,7,8,9,10,12, positive results as *S. typhimuirim* spp. Lane 2,3 5,13negative result , lane M 2000bp marker (ladder).

To cite this paper: Saeed A.A., Hasoon M.F. and Mohammed M.H. 2013. Isolation and molecular identification of Salmonella *typhimurium* from chicken meat in Iraq. *J. World's Poult. Res.* 3(2): 63-67.
Journal homepage: http://jwpr.science-line.com/

Table 4. Results of detecting *salmonella spp*. by single plex PCR *16s rRNA* gene and *in vA* gene.

Sa Sample origin			Single plex PCR Detect *16s r RNA* gene			Single plex PCR detect *inVA* gene		
Origin	**trademark**		**No. tested sample**	**No. of positive**	**%**	**No. tested sample**	**No. of positive**	**%**
Jordan	chicken jd		5	4	80	4	1	25
Turkish	casken oglo	tu	4	3	75	3	0	0
Brazil	al-kafeel		4	4	100	4	1	25
India	al-murad	i	6	6	100	6	3	50
U.S.A.	thighs	u.	6	6	100	6	2	33.3
Total	T		25	23	92	23	7	30.4

Discussion

Salmonellosis is considered one of the anthropozoonotic disease of a serious medical problem and raises great concern in the food industry. Poultry is the most potential source of *Salmonella* food poisoning in man (Ailsa, et al., 2003).

In the present study the prevalence of *Salmonella spp* based on Tetrathionat broth as enrichment media were 55 % (55/100) this results came compatible with (Vera, et al., 2005) which his result (58.6%) from chicken meat when used Tetrathionat broth as pre enrichment media 42 ˚C, and higher than those obtained by **(**Pietzsch, et al., 1984) (48%.**)** and (Arroyo et al., 1995) (31.4%) the difference in the results may be attributed to difference in sampling procedure. Several bacteriological selective media have been used to isolating *Salmonella spp.* like bismuth sulphate agar and the results of isolation were 60 % (33/55) and this finding higher than (Dhaher, et al., 2011) when use Bismuth sulphate agar to isolated Salmonella from a ported chicken in market of Baghdad city which his results was 24.76%. Other chromogenic agar was used as one of the latest techniques that used in recent decade to rapid isolation of pathogenic agent in water and food (Tavakoli et al., 2008*), Salmonella* spp. was isolated (29/33) samples with percent (87.8 %) which was significantly higher what has been reached in the study (Nancy et al 2005), the reason of this variation due to the difference in the number of samples examined and health standards in the massacres.

The present study shows that the total percentage of isolation *Salmonella spp*. according to the reading of API 20-E system were 25 isolates from 27 with percentage 92.5% and this percentage was very closer to (Nucera et al., 2006) that was his result 99% when evaluated API 20-E as indicator for *Salmonella enterica*.

In this work molecular genetics study has been carried out to identify the genetic characters of *Salmonella* by using of *16s r RNAgene* or *invA gene* specific PCR (White et al., 2002), the results showed that chicken meat samples were 92% (23/25). These results obtained were in corroboration with (Raafat et al., 2011; Darwin et al., 1999). The high relationship found between isolates from chicken meat and patient with food poisoning signs indicates a close genetic relationship between *Salmonella* isolation of *Salmonella typhimurium* from poultry meat compared to that isolates from human.

Chicken meat inspection for *Salmonella spp* should be under supervision of Public Health and Veterinary Authorities to ensure the detection of the spread of zoonosis and identify the prevalence in human to improve preventive measures and decrease contamination of poultry products

REFERENCES

Ailsa, D.H. 2003.Food borne microorganism of public health significance. 6th ed.,AIFST.209-255.

Arroyo, G. and Arroyo, J.A. 1995. Efficiency of different enrichment and isolation procedures for the detection of Salmonella serotypes in edible offal. Journal of Applied Bacteriology 79: 360–367.

Baay M.F. and Veld, J.H. 1993. Alternative antigens reduce cross-reactions in an ELISA for the detection of *Salmonella enteritidis* in poultry. J Appl Bacteriol 74: 243-247.

Bailey, JS and Cosby, DE. 2003. Detection of *Salmonellae* from chicken rinses and chicken hot dogs with automated Bax PCR system. J. Food Protect 66: 2138-2140.

Carraminana, JJ, Rota, C. Agustin, I. and Herrera, A. 2004. High prevalence of multiple resistance to antibiotics in *Salmonellae* serovars isolated from a poultry slaughterhouse in Spain. Vet. Microbiol 104: 133-139.

Darwin, K.H. and Miller, V.L. 1999. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin. Microbiol. Rev. 12: 405-428.

Dhaher, F. H; Awni, M. N; Mahmood M.M; and Jamil H. S. 2011. Public Health and Food Safety Lab.\ Ministry of Agriculture Isolation and Diagnosis of Salmonella in Animal Origin Food, Import feed in Baghdad Local Markets and Local Poultry Farms 3: 2011.

Kimura, AC; Reddy, V and Marcus, R 2004. Chicken consumption is a newly identified risk factor for sporadic *Salmonellae enteric* serotype *enteritidis* infections in the United State. Clin. Infect. Dis. 38: 244-252.

Nancy, D., Vicki, R., kircher,S.; patty, P.; and Krista sturm 2005. Evaluation of BBL™ chromagar™ salmonella: AOAC performance tested methods diagnostics 7 loveton circle

Nucera, D. M.; Maddox, C. W.; Hoien-Dalen, P. and Weigel, R. M. 2006. Comparison of API 20E and *invA* PCR for identification of *Salmonella enterica* isolates from swine production units. J. Clin. Microbiol. 44(9): 3388–3390.

To cite this paper: Saeed A.A., Hasoon M.F. and Mohammed M.H. 2013. Isolation and molecular identification of Salmonella *typhimurium* from chicken meat in Iraq. *J. World's Poult. Res.* 3(2): 63-67.

Journal homepage: http://jwpr.science-line.com/

Pietzsch, O. and Burse, M. 1984. Media for *Salmonella*. Inter. J. Food, Microbiol., 26: 117-131.

Raafat H., Sohaila F., Hassan, A., Ashraf M.Abd El-Malek; Moemen, A. and Elsayh, K.H.I. 2011. Detection and identification of Salmonella species inminced beef and chicken meats by using Multiplex PCR in Assiut city Vet.world. 4 (1): 5-11.

Soultose N, Koidis, P. and Madden R.H. 2003. Prevalence of *Listeria* and *Salmonellae* in retail chicken in Northern Ireland. Appl. Microbiol. 37: 421-423.

Tavakoli,H., Bayat,M.; Kousha, A.,and. Panahi, P. 2008..The Application of Chromogenic Culture Media for Rapid Detection of Food and Water Borne Pathogen American-Eurasian J. Agric. & Environ. Sci. 4 (6): 693-698

Varnam, AH 1991. *Foodborne pathogens*. 1st. Edn., Wolfe Publication Ltd., PP: 71-76

Vera, L.M.; Ricardo R.; Lina, C. A.; Silva, M.G. 2005. evaluation of three enrichment broths and five plating media for *salmonella* detection in poultry Brazilian Journal of Microbiology 36: 147-150

White, P.; Meglli, K.; Collins, D.; and Gormely, E. 2002. The prevalence and PCR detection of Salmonella contamination in raw poultry. Vet. Microbiol. 89: 53

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