Mesopotamia Environmental Journal **ISSN 2410-2598**
Mesop. environ. j. 2016, Spicial Issue A.;33-38
(proceding of 6th International conference for Environmental Sciene –University of Babylon).

Bacteriological and Molecular detection of *Salmonellatyphimurium* from Chicken Meat in AL- Najaf province

Angham Jasim Muhammed Ali[1] Haqee Abd.Al.Abaas Essa[2] Amer A. Al-Ramahi[3]

[1,2,3] Institute of pathology analysis, University of Technical
Corresponding Author: Haki196200@gmil.com

To cite this article:

Ali,A.J.M.;Essa,H.A.;Al-Ramahi,A.A.Bacteriological and Molecular detection of Salmonellatyphimuriumfrom Chicken Meat in AL-Najaf province. *Mesop. environ. j*., 2016, Spicial Issue A.;33-38.

Abstract

This study was conducted to detect the prevalence of Salmonellae infection among isochicken meat samples imported from different area to local markets in Najaf governance. The result showed that 11 and 13 isolates were belong to Salmonella spp. According to identification by Biochemical test and vitek system respectively , where as the result of identification by PCR using 16s , ,RNA and inVA gens showed that only 17 and 8 isolates were belong to Salmonella spp respectively . out of 25 Salmonella spp. Isolates that detected by PCR only 10 isolates were belong to Salmonella typhimurium.the highest percent age of isolates were 85.8 % for foreign origin and the lowest percent age were 23% from local origin.

Keywords: Meat, S.typhimurium, PCR.

Introduction

Poultry meat is the combination of muscle tissue, attached skin, connective tissue, and edible organs of avian species commonly used for food. Chicken meats comprise about two-thirds of the total production in the world[1].

Several nutritional factors such as high level of protein and low fat content and favorable content of unsaturated fatty acids contribute to the popularity of poultry meat, of which sensory, dietary and economic factors are important. Poultry meat is easy to prepare at home and widely used in restaurants and fast-food establishments. There is no primary religious restriction on the consumption of poultry meat[2].

Poultry products have always topped the incidence of salmonellosis in many developing countries including India, Egypt, Brazil and Zimbabwe[3]. Contamination with Salmonella in poultry products can occur at multiple steps along the food chain, which includes production, processing, distribution, retail marketing, handling and preparation[4].. Salmonellatyphimurium are the most predominant isolated organisms in most S. typhimurium cases associated with the consumption of contaminated poultry, pork and beef products[6]. The S. typhimurium isolates are commonly found to be

antimicrobial resistant, and to evaluate the analytical methods currently used for identifying these emerging strains, in particular to advise whether the public health risk, when detecting these strains in animals or food, should be considered similar, more or less important than (other) S. typhimurium strains[7]. PCR based on oligonucleotide primers called m-PCR has been developed which is more quickly and sensitive than bacterial culture[8].

Therefore the objective of this study is to detect the prevalence of S. typhimurium in frozen raw chicken meat to take care during cooking and consumption of these products and confirmation of isolates using PCR.

Material and Methods

Sample collection

Chicken samples were collected from different market in AL-Najaf city with different origin include different trademark (local and foreign Chicken,) about 25 g of meat sample were placed in enrichment medium tetrathionate broth and then transported to microbiology laboratory for 18-24 hr at 37°C.. This study was occurred during the period from December 2014to June 2015.

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.(9)

Specific Primers Sequence Used for PCR Amplification

The primers used for the detection specific sequence of *16s rRNA*gene ribosomal genes of *Salmonella* spp F (CGG,ACG,GGT,GAG,TAA,TGT,CT)and,R (GTT,AGC,CGG,TGC,TTC,TTC,TG) with size product 406bp(10)and *invA*gene encoding proteins of a type (T3SS) III secretion system F ATG,CCC,GGT,AAA,CAG,ATG,ATG,AGR,CTC,GCC,TTT,GTC,GGT,TTT,AGR with size product 558bp.(11).These primers were specific for designed in this study by using NCBI Gene Bank and Primer online and provided by (Bioneer company, Korea) .

DNA extraction

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

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

For the detection of *S. typhimurium*by PCR, the PCR amplification mixture consist of 5 µl of PCR PreMix [(Bioneer Korea.)contain: 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] , 5 µl of template DNA, 1.5 µl of each forwarded and reversed primers and 7. Ml PCR water to complete the amplification mixture to 20 µl. The thermocycler condition were controlled as following for both 16s rRNA and invA genes: initial deneturation at 95ºC for 1 min. and 30 cycles of 95C˚ for 5min ;55ºC for 30 s at 55 C˚ and 72 C˚ for 45swith final extension at 72ºC for 7 min.

Electrophoresis:

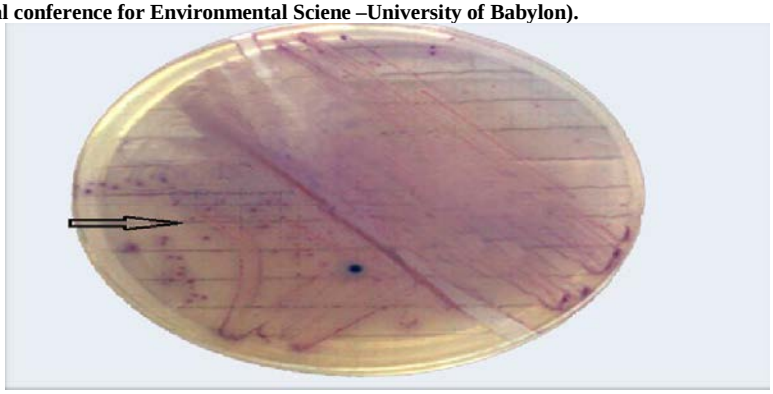
The PCR products resulted from amplification of*Salmonella spp*. specific-PCR were analyzed with electrophoresis on 1% agarose gel stained with ethidium bromide and visualized by UV illumination. The amplification were compared with DNA marker (2000 bp).

Results and Discussion

Culture characters

The total percentage of isolation on tetrathionate broth, bismuth sulphate agar, chromogenic agar was 65.2% (65/150), 50 % (75/150, the highest percent age of isolation was India than localorigin. The colonies of *Salmonella spp.*on chromogenic agar were variable in size convex and mauve in color.

Mesopotamia Environmental Journal ISSN 2410-2598
Mesop. environ. j. 2016, Spicial Issue A.;33-38
(proceding of 6th International conference for Environmental Sciene –University of Babylon).



(Figure 1)*Salmonella spp*.or*S. tyhimurium* on chromo Salmonella agar.

Identification of *S.typhimurium* byVitek system

Salmonella isolates were showed positive productive results to H2S, TSI, SIM and gives negative for indole, VogsProskauer and ureas. The total percentages of these tests were 44.2% (10 \ 25).While the result of Vitek showed that 13 isolated positive from 25 with percentage 53.5% as in(table 1).

Table 1. Detection of *S. typhimurium* byBiochemical test and Vitek system

	Biochemical test			**Vitek system**	
Origin	**No. of tested sample**	**No. of positive**	**(%)**	**No. of positive**	**(%)**
A	5	3	63.8	2	45
B	4	2	50	1	25
C	6	3	50	2	33.3
D	4	2	50	3	63.8
E	6	2	33.3	2	45
Total	25	12	53.5	10	44.2

A:India origin :B:U.S.A C:Turkish D:al-kafeel E:Al Kathem

Detection of *S. typhimurium* by PCR

The result of PCR showed that 17(68%) isolates were belong to*S. typhimurium by* appearing of amplican with 406 bp by amplification of 16s , RNA and 558bp by amplification of invA gene.
The total percentage for isolation of *S. typhimurium* from chiken meat was 68 % .The highest percentage was from Alkafeal meat using*16s rRNA* compred with 50% using invA gene. (Table 2).

Table 2.detecting of*S. tyhimurium* by PCR technique.

		Detecting by16s r RNA		Detecting byinvA	
Origin trademark	**No. tested sample**	**No. of positive**	**%**	**No. of positive**	**%**
A	5	3	45	2	20
B	4	1	25	0	0
C	4	2	50	1	25
D	6	3	50	3	50
E	6	1	10	2	33.3
Total T	25	10	40	8	30.4

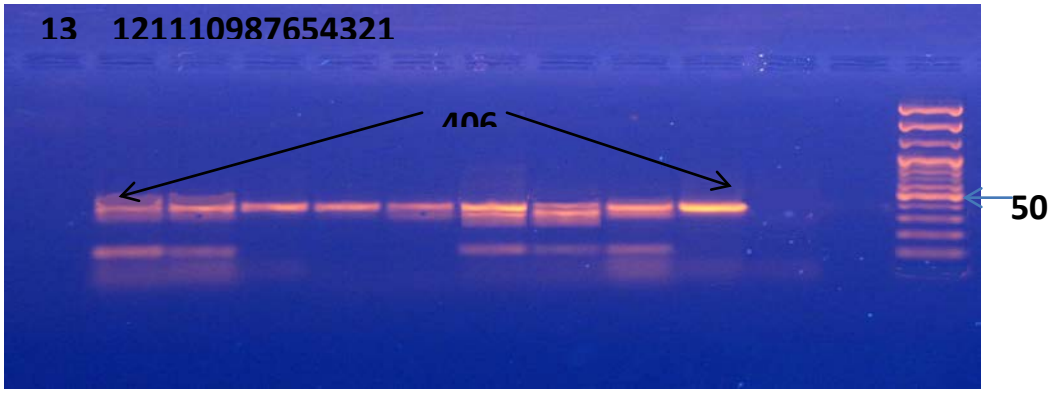


Figure 2. agarose gel electrophoresis for amplification of 16s rRNA gene (406bp) of *Salmonella* spp. lane 1 , lane 2,3,13 negative results ,lane 3,4,5,6,7,8,9,10,12, positive results.

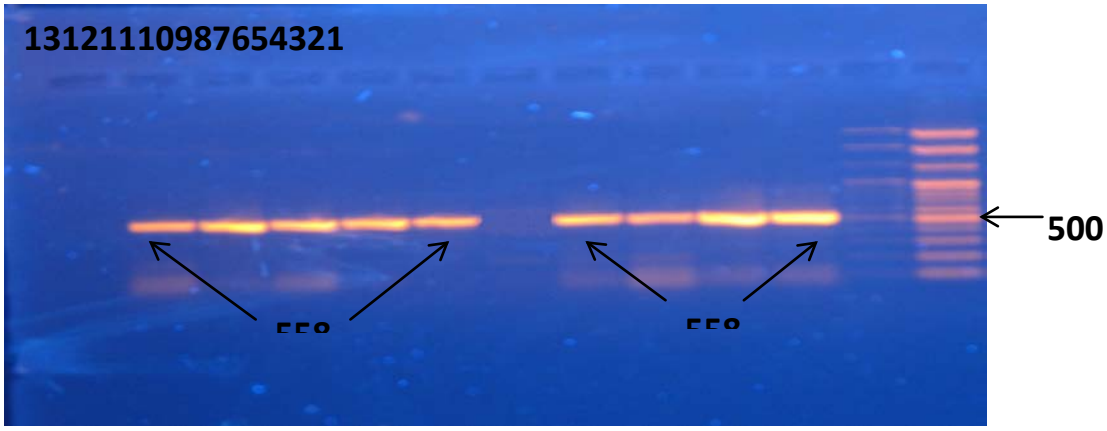


Figure 2. agarose gel electrophoresis for amplification of invA gene (558bp) of *Salmonella* spp,lane 3,4, 6,8,9,10,11,12, positive results as *S. typhimuirum*spp. Lane 2,7,13negative result .

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 (12).

In the present study the prevalence of *Salmonella spp*based on Tetrathionat broth as enrichment media were 65.2% (65/150), 50 % (75/150 for both origion,this results came compatible with (13) who isolates(58.6%) of *Salmonella*from chicken meat when used Tetrathionat broth as pre enrichment media 42 ˚C and higher than those obtained by **(**14) (48%) and (11) (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 agree with finding of (14) when use Bismuth sulphate agar to isolated *Salmonella* from a ported chicken in market of Baghdad city which . 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 (12,15)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 isolation of*Salmonella spp*. according to the reading of vitek system were 12 isolates from 25 with percentage 92.5% and this percentage was very closer to (16) that was his result 99% when evaluated vitek 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* (10) , the results showed that chicken meat samples were 92% (23/25). These results obtained were in corroboration with (17). 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.

References

[1] Ruban, S.W, Thiyageeswaran M, and Sharadha R. Isolation and identification of *Salmonella* spp. from retail chicken meat by polymerase chain reactio1` n. Int J Microbiol Res. 1(3):106–109. 2010.

[2] Mulder, R.W.A.W. Hygiene during transport, slaughter and processing. In: Richardson RI, Mead GC, editors. Poultry meat science: Poultry science symposium series. Vol. 25. Massachusetts: CABI Publishing. pp. 277–283. 1999.

[3] Yang, B.,Xi,M. Wang X., Cui S. Yue T,and Hao H. Prevalence of *Salmonella* on raw poultry at retail markets in China. J Food Prot. 74(10):1724–1728. 2011.

[4] Dookeran M.M. Baccus-Taylor ,G.S, Akingbala J.O., Tameru B,and Lammerding A.M. Transmission of*Salmonella* on broiler chickens and carcasses from production to retail in Trinidad and Tobago. J AgricBiodivers Res. 1(5):78–84. 2012.

[5] Center for Disease Control and Prevention Multistate outbreak of human *Salmonella typhimurium*infections linked to live poultry. Atlanta: Center for Disease Control and Prevention. [Online] . [Accessed on 27 April, 2013].

[6] Vose, D., Koupeev, T.; Mintiens K. A quantitative microbiological risk assessment of *Salmonella* spp. in broiler (*Gallus gallus*) meat production.Technical report submitted to EFSA. 2010.

[7] EFSA Panel on Biological Hazards Scientific opinion on monitoring and assessment of the public health risk of "*Salmonella typhimurium*-like" strains. EFSA J., 8(10):1826. 2010.

[**8] Salehi, T.Z.; Gharagozlou, M.J.; Shams N; Madadgar, O., Fasaei BN, and Yahyaraeyat R.** Molecular characterization of a *Salmonella typhimurium* isolate from Caspian pony. Iranian J Biotechnol. 10:49–53. 2012.

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

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

[11] Bailey, J.S. and Cosby, D.E. Detection of *Salmonellae* from chicken rinses and chicken hot dogs with automated Bax PCR system. J. Food Protect 66: 2138-2140. 2003.

[12] Ailsa, D.H. Food borne microorganism of public health significance. 6th ed.,AIFST.209-255.2003.

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

[14] Dhaher, F. H; Awni, M. N; Mahmood M.M; and Jamil H. S. 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.

[15] Tavakoli,H., Bayat,M.; Kousha, A.and. Panahi, P. 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. 2008.

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

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