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Epidemiological and Molecular Studies of *Salmonella* Isolates from Chicken, Chicken Meat and Human in Toukh, Egypt

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Abstract: *Salmonellosis* is one of the most important zoonotic bacterial pathogen of food-borne infection all around the world.The present study was carried out to report the prevalence of the serotypes and genetic types of *salmonella* among broiler chickens,raw chickens meat and patients suffer from food poisoning signs in Toukh Egypt.Samples collected from (50) diarrheic broiler chicken,(50) raw frozen chickens meat and (30) diarrheic patients with food poisoning signs were bacteriologically and serologically processed for identification of *Salmonella*. Isolates were subjected to multiplex-PCR using specific *Salmonella* primers.The prevalence *of Salmonella* spp was 7(14%), 2(4%) and 3(10%) in chickens,raw chickens meat and patients respectively. The*Salmonella* Isolates were serologically identified as 7(58.33%) and 5[41.66%] *S.enteritidis* and *S.typhimurium* respectively.The antigenic formula of serovar *S.enteritidis* has somatic antigen O: 1,9,12 and phase I (g,m) and phase II [1,7] flagellar antigen (H),While serovars *S.typhimurium* have the O: 1,4, (5),12and phase I and phase II.Multiplex-PCR yield similar diagnostic amplified DNA bands of molecular size marker at 250bp in tested *S.enteritidis* and 620bp in examined *S.typhimurium*,indicating the zoonotic potential of the organism and the role of chicken and chicken meat as sources in the epidemiology of the human *salmonellosis*.

Key words: *S. typhimurium* % *S. enteritidis* and Multiplex-PCR

INTRODUCTION

Salmonellosis is a serious zoonotic food-borne pathogen which causes outbreaks and sporadic cases of gastroenteritis in human world wide [1] chickens have been implicated as a major source of *Salmonella* contaminated food products as chicken eggs or meat that cause human *salmonellosis* [2]. In Egypt *S. enteritides* were isolated from broiler chicken, chicken meat and food poisoning patient [3] The clinical illness characterized by fever, nausea and diarrhea, vomition and abdominal pain after an incubation period of only 12 to 72 hrs [4]. *Salmonella* m.o. can pass through the food chain from feed to poultry and finally human occasionally causing *salmonellosis* [5]. The serotype associated most frequently with this diarrheal disease in United States is *S. typhimurium* [6]. In Europe the predominant serovar of *S.typhimurium* was monophasic (phase I) serovar O:4,12:d among 377 broiler flocks [5]. For the effective prevention and control of this food-borne zoonosis rapid and sensitive detection methods are required [7]. Conventional diagnostic methods are laborious and time consuming. Molecular detection can improve the time to reporting of the final result from several days to the next day [8]. Multiplex-PCR assay may be available tool in epidemiological investigation and surveillance by relating isolates from different sources to a common origin [9]. The present study is based on analytical epidemiological investigation and molecular typing by using multiplex-PCR in addition to traditional phenotypic analysis such as biotyping and serotyping,objective to identify the epidemiological and genetic relationship of *Salmonella* isolates from chicken, chicken meat and patient with food poisoning signs.

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MATERIALS AND METHODS

Samplying: Faecal swab were collected from 50 broiler chickens suffer from diarrhea in different farms in Toukh Kaliobia governorates, 30 raw frozen chickens meat were aseptically collected from markets. As well as 30 stool samples were collected from patients with food poisoning signs (Fever, vomition, nausea, abdominal painand diarrhea) admitted in Toukh hospital. The collected samples were labeled and directed to the laboratory for bacteriological examination.

Isolation and Identification of Salmonella: Each faecal sample and about 25gm of aseptically triturated meat sample were pre-enriched in 250 ml of bufferd peptone water (PBW) as described by [10]. After incubation at 37°C for 18hrs about 0.1ml of the pre-enriched culture was inoculated into 10 ml of selenite f-broth (Oxoid) and incubated at 43°C for 18-24 hrs Therafter, a loop full of each broth was streaked on *Salmonella-Shigella* agar (S.S. agar) and Xylose Lysine Desoxycholate agar (XLD-agar) and incubated for 24 hrs at 37°C. Colonies of typical growth were picked up and confirmed morphologically according to [11] and biochemically by applied scheme of [12] such as catalase, oxidase, H2S production on TSI agar, indol production, methyl red,Voges Proskauer,citrate utilization,urease,gelatin liquifaction and sugar (dextrose, lactose, sucrose, mannitol and maltose) fermentation tests.

Serotyping of Isolates: Biochemically identified *Salmonella* isolates were serotyping according to Kauffmann White Scheme [13] by slide agglutination test polyvalent (0)*Salmonella* antiserum positive colonies were tested using monovalent (O) and (H) *Salmonella* antisera. Tested isolates were kept at 70°C in brain-heart infusion broth (DIFCO) with the addition of 20%(V/V) glycerol. An aliquaot of this storage solution was taken and incubated in 5ml phosphate-buffered peptone water for 24 hrs at 37°C prior extraction of DNA.

Isolation of DNA: The serologically identified isolates were centrifugated at 8,700 Xg for 15 min.and the cell pellets were re-suspended in 1ml of sterile water. The resuspended cells were re-centrifugated at 12,500Xg for 15min. The pelleted cells were then used for DNA extraction as followed by manufacturer instructions for QIAamp DNA miniprep kit.

Multiplex-PCR Assay: Multiplex-PCR (m-PCR) was done for identification of *Salmonella* serotypes according to [14] by using two pairs of primers. The first primer was used to amplify 250bp of S.enteritidis, was designed as forward S 1-F(2) 5'-GCC GTA CAC GAG CTT ATA GA-3' and reverse S4-R(2) 5'-ACC TAC AGG GGC ACA ATA AC-3'.

The second primer had been done to amplify fragment 620 bp from *S.typhimurium*,was arranged as forward FLi: 15-F(3) 5'-CGG TGT TGC CCA GGT TGG TAA T-3' and reverse . FLi 15-R(3) 5'-ACT GGT AAA GAT GGC T-3'.The specific primers were supplied from Jena, Bioscience,Germany. A100bp DNA was used as molecular size marker.

Multiplex-PCR Procedure: The multiplex-PCR reaction contained 5 ul of template DNA, 0.4 uM of each primer, 10 uM of deoxynucleotide triphosphates, 1.25mM of $MgCL_2$ (25 mM), 2.5U of Tag polymerase, 5 ul of 10-PCR buffer (Promega Crop. MI, USA), and water to bring the final reaction volume to 50 UL. For data analysis, PCR assay was performed in thermal cycler (Techene Tc-512UK).The DNA denaturated for 5min. at 95°C. The mixture was then subjected to 35 cycles at denaturation at 95°C for 1 min. annealing of primer at 50°C for 1 min, and primer extention at 72°C for 2 min. The final extention was held at 72°C for 10min. PCR products were analysed in electrophoresis in agarose gel and visualized in ethidum bromide. The data analyzed by using Gelpro analyzer V4.

RESULTS

Table 1 declared the prevalence of *Salmonella* spp in the collected samples was 12(9.23%), 7(14%) in broiler chickens, 2(4%) raw frozen chickens meat and 3(10%) patients with food poisoning signs. Identification of*Salmonella* spp based on conventional bacteriological and biochemical characters Table 2. Serotyping of isolates were established using specific*Salmonella* antisera (Table 3), 7(58.33%) and 5(41.66%) were identified as*Salmonella enteritidis* and *S.typhimurium* respectively.*S.enteritidis* serovar has the same somatic antigen O: 1,9,12 and phase I : g,m and phase II : [1,7] flagellar antigen,while serovar *S. typhimurium* has O:1,4,(5), 12 and phase I and II, Table 4. The result of multiplex-PCR using 2 primer pairs (S1-F,S4-R) and (FLi 15-F,FLi 15-R). Figure 1. presence of *Salmonella* were documented by the appearance of amplified DNA

Table 1: The prevalence of *Salmonella* isolated from chicken, chicken meat and human

	Samples	Number No	Positive Samples %
1-Broiler chickens	50	7	14
2-Raw frozen chickens meat	50	2	4
3-Patients with poisoning signs	30	3	10
Total	130	12	9.23

Table 2: Biochemical characters of suspected Isolates of *Salmonella spp.*

Biochemical character	Results
Catalase	+
Oxidase	-
H2S production on TSI agar	+
Indol production	-
Methyl red	+
Voges Proskauer	-
Citrate utilization	+
Urease	-
Gelatin liquefaction	-
Sugar fermentation	
Dextrose	+
Lactose	-
Sucrose	-
Mannitol	+
Maltose	+

Positive (+)
Negative(-)

Table 3: Serotyping of *Salmonella* isolates from chicken, chicken meat and human

		Samples No	No. of isolates %	S.enteritidis Positive No	S.typhimurium Positive %
Broiler chickens	7	4	57.14	3	42.85
Raw frozen chicken meat	2	1	50	1	50
Patients with food poisoning signs	3	2	66.66	1	33.33
Total	12	7	58.33	5	41.66

Table 4: Antigenic formula of *Salmonella* serotypes isolated from the examined samples

	Salmonella serotypes Somatic(6) Phase I	Antigenic formula Flagellar (H) Phase II
S. enteritidis	1,9,12	g,m [1,7]
S. typhimurium	1,4,(5),12	I 1,2

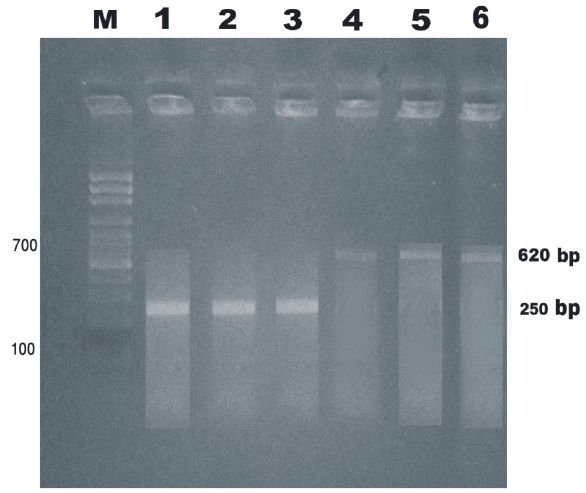

Fig. 1: Showed that multiplex-PCR amplification products of isolated *S. enteritidis* and *S. typhimurium.* Lane M: a 100bp molecular size marker. Lane 1,2,3 *S.enteritidis* at 250 bp in chickens, chickens meat and human respectively. Lane 4,5,6 *S. typhimurium* at 620 bp in chickens, chickens meat and humans respectively.

fragments of *S. enteritidis* at 250bp in all examined isolates, while serovar *S. typhimurium* isolates showed similar DNA bands of the same molecular size at 620 bp in all tested samples.

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 [15]. In the present study the prevelance of*Salmonella spp* based on bacteriological and biochemical characters was found to be 7(14%) in broiler chicken 2(4%) in raw frozen chicken meat and 3(10%) patient with food poisoning signs. Our finding were nearly similar to *Salmonella* spp isolated from 14% broiler chicken in Assiut, Egypt [16], while higher than those isolated from 8% Turkey in Albania [17], lower than those recorded from 23% poultry products in Belgian retail markets [18] and lower than those recently reported in 6(12.5%) patient with food poisoning signs [3]. The difference in the results may be attributed to difference in sampling procedure, locality and difference in method used [19] Serotyping of *Salmonella* isolates identified as 7(58.33%) *S. enteritidis* and 5(41.66%) *S. typhimurium.* Our result nearly simulate previous study in broiler chicken the incidence of *S. typhimurium* was 60% and

86% in England and Spain respectively [20]. In Egypt the predominant serotype differs from one geographic area to another. In broiler chicken identified *5 Salmonella* strains as *S. typhimurium* in Alexandria [21] and serotyped as *S. enteritidis* and *S. typhimurium* in Assiut [15]. While in chicken meat and patient with food poisoning signs identified as *S. enteritidis* [3]. On other hand poultry meat incriminated in *S. typhimurium* infection in India [7] and *S. enteridis* in Iran [22]. This may be due to contamination during its production, handling, packing and storage. The antigenic formula of *Salmonella* serotypes isolated from broiler chickens, raw frozen chickens meat and patients with food poisoning signs showed undistinguishable formula in all examined isolates as *S. enteritidis* has somatic antigen O: 1,9,12 and phaseI:g,m and phase II : [1,7] flagellar antigen, while serovar *S. typhimurium* has O:1,4,(5),12 and phase I,II.These results coincide with antigenic formula of *Salmonellae* as described by [23]. Monophasic (phase I) serovar *typhimurium* O :4,12 : d responsible for Norwegan patients [24] and isolated from German broiler flocks in Denmark and United Kingdom,suggesting that chickens are an important reservoir of human serovar *typhimurium* infection [5]. Molecular typing of *Salmonella* isolates has been useful in investigation the role of poultry in the epidemiology of human *salmonellosis* [25], has a great effect as an epidemiological tool in outbreak of *salmonellosis* [26] and used as a rapid and sensitive technique in detection of *Salmonella* contamination in poultry eggs and meat [7]. In this work molecular genetic study has been carried out to identify the genetic characters of *Salmonella* serovars isolated from broiler chicken,raw frozen chicken meat and patient with food poisoning signs. Multiplex-PCR gave positive result in all *Salmonella* strains where DNA indistinguishable from one another and PCR yielded similar amplified DNA bands of the same molecular size marker at 250 bp in all tested S.enteritidis and 620 bp specific diagnostic bands in all examined *S. typhimurium.* These results obtained were in corroboration with [14,27]. The high similarity found between isolates from broiler, chicken meat and patient with food poisoning signs indicates a close genetic relationship between *Salmonella* serovars isolates from chicken and chicken meat compared to that isolates from human. Previous investigation reporting genetic relationship of serovar *typhimurium* from humans [21], poultry meat [7] and broilers flocks [5]. Thus our results reinforce previous investigations, suggesting that poultry and poultry meat are important sources of human *salmonellosis*.*Salmonella* needs special concern in the developing world because of poor hygienic conditions that favour its spread. *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.

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