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ISOLATION AND IDENTIFICATION OF SALMONELLA SPP FROM CHICKEN MEAT IN KURDISTAN REGION

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Abstract

Salmonella is a bacterial diseases that caused by many strains of salmonella. Salmonellosis is a disease occur in human and chicken, salmonellosis in poultry sometimes causes economic losses, also international trade can be affected if the salmonella presents in poultry products or poultry meat as in this case the infection can transmit to humans. Salmonella is a considered the most important and serious pathogen of bacteria that responsible of food borne infection all over the world. In Kurdistan region, Erbil city the common shapes of poultry are broilers and backyard. This study was done to inform the impact of salmonella epidemic chicken and the prevalence of salmonella's genetic types and serotypes among broilers and raw meat of chickens in Kurdistan, Iraq. Samples were collected from about fifty broiler chickens that suffered from diarrhea, fifty raw chicken's meat and "30" patients that had diarrhea and signs of food poisoning. The serological identification of salmonella after isolation was "58.33%" salmonella enteritidis and "41.66%" salmonella typhimurium. Serotype salmonella enteritidis antigenic formula has "O" somatic antigen "1, 9, 12" and flagellar antigen "H", while the serotype salmonella typhimurium had the "O" somatic antigen "1, 4". The main infection source of humans includes; meat products that comes from consumption of contaminated meat of chicken. Scientifically, the control programs of impact of salmonella control are based on general procedures of hygiene on the campylobacter prevalence at the holding in broiler flocks and at the end of the process of slaughter on the broiler meat.

Keywords: Salmonella, S.typhimurium, Multiplex-PCR, S.enteritidis, Antibiotics, Kurdistan

عزل وتعريف سلالات بكتريا السالمونيلا من لحوم الدجاج في اقليم كوردستان

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الخلاصة

السالمونيلا من الامراض البكتيرية التي تتسبب بها العديد من السلالات، حيث ان المرض يحدث في البشر والدجاج محدثا خسائر اقتصادية في الدواجن وفي بعض الاحيان التجارة العالمية اذا ما ظهرت في منتجات ولحوم الطيور حيث يمكن انتقال الاصابة الى الانسان اذ يعتبر السالمونيلا اهم واخطر المسببات البكتيرية المسؤولة عن العدوى التي تنقلها الاغذية في جميع انحاء العالم. في اقليم كردستان تحديدا في مدينة اربيل الدجاج اللاحم ودجاج التربية المنزلية هي من اكثر انواع تربية الدواجن شيوعا وعليه اجريت هذه الدراسة لمعرفة اصابة الدجاج بالسالمونيلا والانواع الوراثية والانماط المصلية لهذه الاصابة بين دجاج اللاحم ولحم النيء للدجاج المذبوح المستهلكة في هذه المدينة. اذ تم جمع العينات من حوالي 50 دجاجة لاحم مصابة بالإسهال و50 عينة لحم دجاج نيء و30 عينة من اشخاص مصابين بالإسهال واعراض تسمم غذائي. التعرف المصلي على السالمونيلا بعد العزل كان سالمونيلا انتريتيدز 58.33% وسالمونيلا تايفيموريم 41.66%. تحتوي تركيبة المستضد المصلي للسالمونيلا المعوية انتريتيدز على مستضد جسدي "1.9.12" O ومستضد سوطي "H", بينما يحتوي المستضد سالمونيلا التيفيموريم المصلي "O" الجسدي "4.1". المصدر الرئيسي للإصابة بالعدوى في الانسان يتضمن: منتجات اللحوم التي تأتي من استهلاك لحوم الدجاج الملوثة بهذه البكتريا وعليه من الناحية العلمية تستند برامج التحكم في مكافحة تأثير السالمونيلا على الاجراءات العامة للنظافة للسيطرة على انتشار الكومبايلوبكتر في قاعات التربية للدجاج اللاحم والسيطرة على لحم الفروج في نهاية عملية الذبح.

كلمات مفتاحية: السالمونيلا، سالمونيلا تايفيموريم، متعدد تفاعل البلمرة المتسلسل، سالمونيلا انتريتيدز، المضادات الحيوية، اقليم كوردستان.

Introduction

The most serious food borne pathogen which is zoonotic causes gastroenteritis in sporadic cases in humans all over the world, the infection of salmonella in poultry can be a non-host specific infection or a host specific infection (10). The major source of salmonella is detected to be chickens and also the contaminated products of food, like meat or eggs of chicken which lead to human salmonellosis (9). Chicken meat and broiler chicken and patients can show salmonella enteritidis after the isolation (1). People who catch salmonella from broiler meat can show some clinical signs like, diarrhea, fever, nausea, abdominal pain and vomiting, the incubation period of salmonella in human is about 12 to 72 hrs (5). Salmonella microorganisms affect the

broilers then through food chain it can transmit to humans to cause human salmonellosis (3). The diagnostic methods which are conventional are time consuming but the molecular detection methods can consume little time in the comparison to the conventional methods as the results and reports in the molecular methods can improve in several days (14). The assay of multiplex PCR is considered a tool in surveillance by connect the different isolates from different sources to the common origin. The effects of salmonella types on chicken is very difficult, for example; salmonella typhimurium lipopolysaccharides that have cell wall components of Gram –ve bacteria can affect the chicken through the immunological changes and neuroendocrines (13). In the field of drugs and antibiotics, salmonella detected to be sensitive completely to Amoxicillin, Cefotaxime and cefadroxil and to be resistant to Levofloxacin (5).

Materials and Methods

The process of sampling: Collected fecal swabs and cloacal swabs (done by inserting a swab gently into the vent from live bird or vigorously inserting the swab to make swabbing the mucosal wall from dead bird and this swabs should be stained deeply with fecal materials) from about fifty broilers that were suffering from diarrhea. Also collected raw frozen meat of chicken about fifty that were collected from backyard chickens. And "30" samples taken from human's stool mainly from patients that had signs of food poisoning with diarrhea that admitted from privet hospital, these samples from humans were labelled then sent them to the bacteriological lab for an accurate "bacteriological examination".

Isolation of salmonella and identification of it: Pre-enrichment of all fecal sample and triturated meat sample which is about 25gm in 250 ml of BPW (buffered peptone water) as mentioned. Then, incubation occurs for 18 hrs at 37°C, then, we take 0.1 from the previous pre-enriched culture to inoculate it in selenite f-broth (10ml), then it incubated for 18:24 hrs at 43°C then, take a good loop that filled well of each broth then streaked it on S.S agar (salmonella -shigella agar) and XLD agar (Xylose Lysine deoxycholate agar) to incubate it at 37°C for about 24 hrs. Typical growth colonies were picked up and examined morphologically, also the biochemical characters examined through applied scheme like; H2S, oxidase, catalase, indol production, voges proskauer, TSI agar, methyl red, citrate utilization, gelatin liquefaction, urease, and sugar fermentation tests.

Serotyping of isolates: The identification of salmonella isolates biochemically were serotyping depending on the scheme of Kauffman white (7). Using the test of slide agglutination polyvalent "O" salmonella antiserum colonies which gives positive were tested by monovalent "H" and "O" antisera of salmonella. The isolated which were tested kept in brain heart infusion broth at 70°C with adding a glycerol 20%, solution stored and incubated for 24hrs at 5ml of phosphate-buffered peptone water at 37°C before extraction of DNA.

DNA isolation: Centrifugation at 8,700 Xg is done for the identified isolates for about 15 min, then, re-suspension the pellets in 1ml sterilized water. Re-centrifugation of

the re-suspended cells is done at 12,500 Xg for about 15 min. DNA extraction is done using these pelleted cells as following by the QIAamp DNA miniprep kit manufacturer instructions.

Assay of multiplex PCR: The assay of multiplex PCR was done for the purpose of salmonella's serotypes identification (11). The identification can be done by using primers (2 pairs). The first one was in action of amplify about 250 bP of salmonella enteritidius, this primer was designed as ((S 1-F (2) 5'-GCC GTA CAC GAG CTT ATA GA-3')) to serve ((S4-R (2) 5'-ACC TAC AGG GGC ACA ATAAC-3')). The second primer is designed in order to build a fragment of 620 bp from typhimurium type of salmonella that is arranged forward ((FLi: 15-F (3) 5'-CGG TGT TGC CCA GGTTGG TAAT-3)) and reverse as ((. FLi 15-R (3) 5'-ACT GGT AAA GAT GGCT-3')).

Procedures of multiplex PCR: The reaction of multiplex PCR contained the following: 0.4 uM of each type of primers, 5 UL of DNA template, 10 uM of DNT (deoxynucleotide triphosphates), 2.5 U of tag polymerase, 1.25mM of MgCL2, 5 UL of promega crop MI, USA (10-PCR buffer) and then add water to reach with the volume to final volume of reaction that was equal to 50 UL. Assay of PCR was done in the thermal cycler for the data analysis, DNA denaturation occurs at 95°C for 5 min. After that the mix subjected to 35 cycles at 95°C at denaturation for 1 min, and the extension of primer at 72°C for 2 min. The final step of extension was done at 72°C for 10 min. The analysis of PCR products is done in agarose gel electrophoresis and could be visualized in the ethidum bromide. Gelpro analyzer V4 was used for data analyzed.

Testing of antibiotic susceptibility: 4 commonly used antibiotics were tested by disk diffusion assay by the interpreting of diameters of inhibition zones around the disks of antibiotics, the tested antibiotics are the following: Amoxicillin (AMC30), Cefotaxime (CTX30), Cefadroxil (CFR30) and levofloxacin (LEV5).

Results

The prevalence of salmonella species is declared in table 1 that collected samples was 7 (14%), 12 (9.23%) in the broilers, and 2 (4%) in the raw frozen meat of chicken and also 3(10%) humans with signs of food poisoning. The salmonella species identification was done by biochemical characters and conventional bacteriological table 2 using the specific antisera of salmonella table 3, 5(41.66%) and 7(58.33%) were identified obviously as salmonella typhimurium and salmonella enteritidis respectively. The serovar of salmonella enteritidis has the "O" somatic antigen "1, 9, 12" and phase one and phase two (10, 13) the salmonella typhimurium serovar has the flagellar antigen "O", "1, 4" and phase one and phase two (3) table 4. Salmonella isolates showed complete sensitivity to antibiotics Amoxicillin, Cefadroxil and Cefotaxime and complete resistant to levofloxacin (figure1).

The multiplex PCR that worked using 2 primers showed a result of salmonella presence by the appearance of DNA that amplified (figure 2).

Table 1 salmonella prevalence that isolates from broilers, raw meat of chicken and human.

	Samples	Number	Positive samples %
1.Broilers	50	7	14
2.Raw frozen meat of chickens	50	2	4
3.Patients with signs of poisoning	30	3	10
Total	130	12	28

Table 2 characters of suspected salmonella spp isolates biochemically.

Characters in biochemical reactions	Results
oxidase	-
Catalase	+
Indol production	-
Production of H2S on the TSI agar	+
Voges Proskauer	-
Methyl red	+
Utilization of citrate	+
Gelatin liquefaction	-
Urease	-
Fermentation of sugar	
Lactose	-
Dextrose	+
Mannitol	+
Maltose	+
Sucrose	-

Table 3 Salmonella serotyping of isolates that taken from broiler chicken, raw meat of chicken and humans.

	Samples number		% of isolates number	Positive number of salmonella enteritidis	Positive % of Salmonella typhimurium
Broilers	7	4	57.14	3	42.85
Raw frozen meat of chicken	2	1	50	1	50
Patients with signs of food poisoning	3	2	66.66	1	33.33
Total	12	7	58.33	5	41.66

Table 4 Antigenic formula of serotypes of salmonella isolates from broilers, raw meat of chicken and humans.

	Serotypes of salmonella Somatic (O) Phase one	Antigenically formula Flagellar (H) Phase two
Salmonella enteritidis "1,9,12"	g, m	{1, 7}
Salmonella typhimurium "1,4,5,12"	I	1 , 2

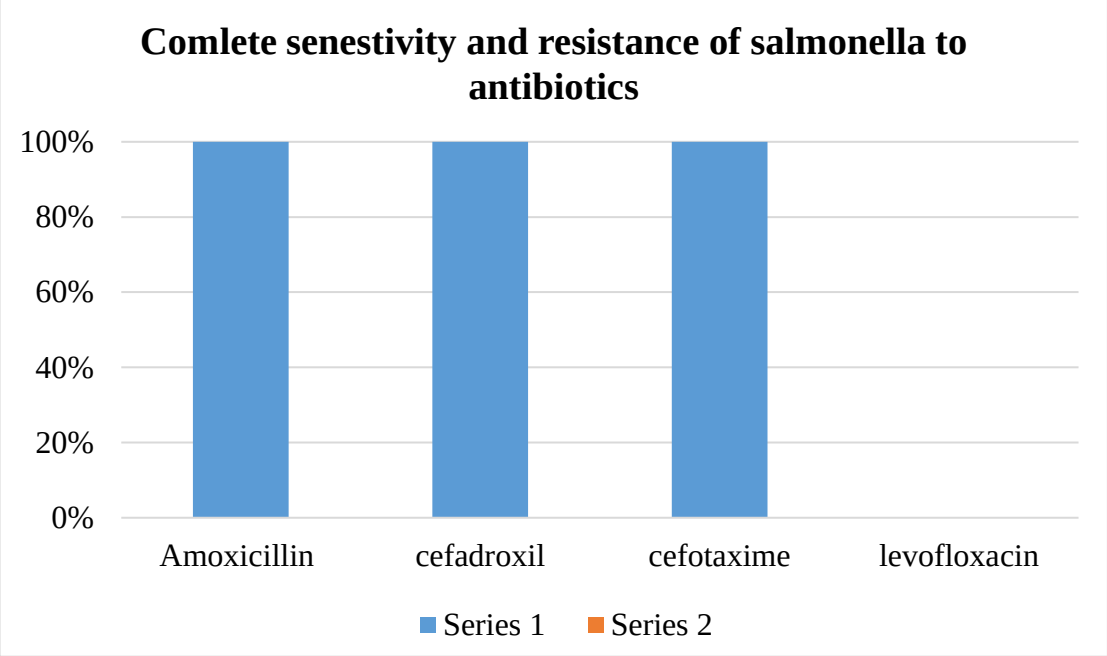

Figure 1 Antibiogram profile of salmonella isolates shows the complete sensitivity and resistance to antibiotics.

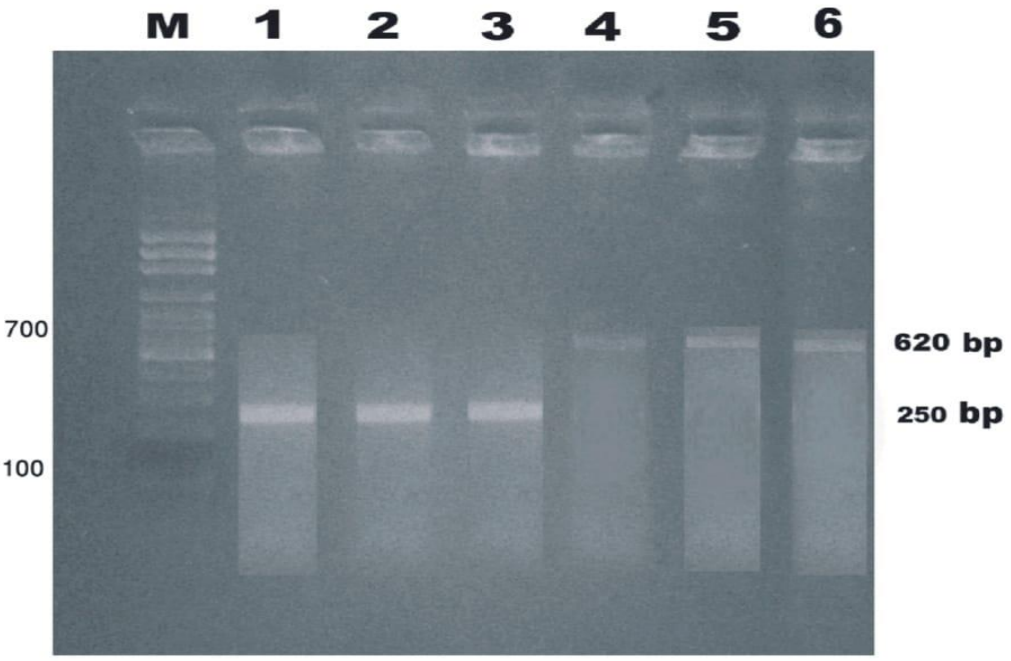

Figure 2 Amplification products of multiplex PCR of isolated Salmonella enteritidis and salmonella typhimurium.

Molecular size of marker Lane: M: a 100bp, lane 1, 2 and 3 in salmonella enteritidis at 250bp in broilers, raw meat of chicken and human respectively. Lane 4, 5 and 6 in salmonella typhimurium at 620 bp in broilers, raw meat of chicken and human respectively, salmonella enteritidis fragments at 250 bp in all isolates that was examined while salmonella typhimurium serovar isolates gave similar bands of DNA with same molecular size in all isolates that was examined at 620 bp.

Discussion: One of the anthropozoonotic diseases is a salmonella which considered as a serious problem in the medical field and affects the industry of food as the most potential source of salmonella is poultry that lead to human food poisoning (6). In our study, salmonella species prevalence based on biochemical and bacteriological characters which found to be 7(14%) in broilers, 2(4%) in raw meat of chicken and 3(10%) in patients that suffered from signs of food poisoning, Salmonella serotypes antigenic formula of isolates that taken from broilers, raw meat of chicken and patients showed undistinguishable formula in the examined samples as salmonella typhimurium has O: 1, 4, 5, 12 and phase one and two, while salmonella enteritidis has somatic antigen O:1, 9, 12 and phase one (g, m) and phase two : (10, 13) flagellar antigen (13). In this study we use molecular genetics to recognize the main characters of salmonella genetically. Multiplex-PCR gave a (+ve) result in every strains of salmonella where the DNA undistinguishable from one another and similar amplified DNA bands were yielded by the PCR and these bands have the same molecular marker size as in salmonella enteritidis it will be 250 bp and in salmonella typhimurium it will be 620 bp. The similarity which found between samples isolated from broilers, meat of chicken and patients indicates that there is a close relationship genetically between the serovars of salmonella isolates (4). Thus our results and discussion reinforce previous investigation that suggesting the bad impact of salmonella epidemic chicken on the economic and food industry also on human health as the broilers and raw meat of chicken are the main source that cause human salmonellosis. So, salmonella needs special programs especially in our country because of the bad hygienic control that increase its spread. Also veterinary authorities and public health should ensure the spread of the epidemic and ensure its

prevalence in human to apply preventive measurements and prevent its spread among poultry and also the products of poultry. People also should know some steps to do at home like; how to handle the raw chicken and how to store it? Also cooking the chicken meat should be done at 165 degrees Fahrenheit and washing all prepping and cooking surfaces, counters, boards of cutting and hands (2). Hands should be washed for at least 20 seconds with warm water and soap before and after handling the raw meat of chicken and also after bathrooms, handling with pets animals and after diapers changing. Food cross contamination should be avoided also by separating the raw meat of chicken from other food always in refrigerators, freezers, grocery bags and grocery shopping cart. At the store you should make raw chicken as the last thing you select and make sure that the products are well refrigerated or well frozen, at home if you will not use the raw chicken for the upcoming 2 days you should freeze it as chicken can be stored in a freezer for about one year, the uneaten chicken can be refrigerated after 2 hours till two or three days (2).

Conclusion: The impact of salmonella epidemic chicken on the economic and food industries and humans health is very serious and dangerous, the prevalence of salmonella serotypes is informed from samples that taken from "50" broilers, "50" raw frozen meat of chicken and "30" patients who suffer from diarrhea and signs of food poisoning, bacteriological and serological applications were admitted for the salmonella identification in the isolated samples that taken from broilers, raw frozen chicken meat and humans. Also multiplex PCR was used using specific primers of salmonella, the recorded prevalence of salmonella at the end of study was 2(4%), 7(14%) and 3 (10%) for raw frozen meat of chicken, broilers and humans that had diarrhea and suffered from signs of food poisoning respectively. The serological identification of salmonella was 5(41.66%) and 7(58.33%) for salmonella typhimurium and salmonella enteritidis respectively. Salmonella isolates showed complete sensitivity to 3 antibiotics (Amoxicillin, cefadroxic and cefotaxime) while showed complete resistance to (levofloxacin). Especial concerns should be taken from veterinary authorities, public health, people and food industry to limit the salmonella infection and prevent its spread by providing a good hygienic control as bad hygiene improve the spread of salmonella and so the prevalence of salmonella will increase to affect the human health.

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