ISOLATION AND IDENTIFICATION OF *SALMONELLA* FROM WHOLE CHICKEN SAMPLES BY CONVENTIONAL CULTURE AND MOLECULAR BASED METHODS

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ABSTRACT

The aim of this study is to evaluate a molecular methods for detection of *Salmonella* in chicken samples and compare them with conventional methods. A total of 250 whole chicken carcasses have been collected. 100 local chickens, 100 imported chickens, 25 local chicken liver and 25 imported livers.. Twenty one (8.4%) out of 250 samples were found to be *Salmonella* spp. by conventional methods, while only 12 samples were confirmed by polymerase chain reaction (PCR). The highest prevalence of *Salmonella* was detected from local chicken carcasses 12 (12%) followed by imported chicken carcasses 8 (8%) and local chicken liver 1 (4%) by conventional methods. On other hand the ratio were declined to 9 (9%) in local chicken , 2 (2%) in imported chicken , 1 (4%) in Local chicken liver by PCR. The isolates were serotyped at the central public health Laboratory . Furthermore, a total of 12 *Salmonella* serotypes were identified by serotyping technique including 11 *S. enteritidis* and one *S. ohio*. Finally, our results revealed that conventional methods for *Salmonella* identification are not adequate for confirmation therefore, PCR is needed for more identification as it's more sensitive and more specific. This study indicates its need of a strict hygienic measure in the process of poultry meat to reduce the potential contamination of the products. Furthermore, continuous monitoring studies are required to evaluate the prevalence and rate of contamination as well as the nature of pathogens involved in the contamination.

INTRODUCTION

Chicken meat is one of the most common food products consumed by the global population [1]. Universally, *Salmonella* is one of the most commonly isolated pathogen from chicken carcases and liver [2]. *Salmonella* infections continue to be one of the challenging infections in both man and animals around the globe in spite of the development of many strict control measures worldwide [3]. Globally, it is estimated to have 93.8 million human cases of salmonella infections each year [4]. *Salmonella*is a large group of enteric bacteria with a broad range of hosts and can cause enterocolitis (salmonellosis), enteric fever (typhoid fever), and septicemia [5]. *Salmonella enterica* serovar *Typhimurium* and *Salmonella enterica* serovar *Enteritidis* are the most frequently isolated serovar from food borne outbreaks throughout the world [6].

Several methods have been developed for the detection, identification and molecular characterization of *Salmonella* species [7]. Conventional culture methods were used for the isolation of *Salmonella* including non-selective pre-enrichment followed by selective enrichment and plating on selective and differential agars. Suspected colonies were then confirmed biochemically and serologically [8].

More recently, a number of alternative methods for the detection of *Salmonella* in foods have been developed, including immune-assays, nucleic acid hybridization and polymerase chain reaction (PCR) techniques [8]. The Polymerase Chain Reaction (PCR) has become a powerful tool in microbiological diagnostics during the last decade. PCR based methods combine simplicity with a potential for high specificity and sensitivity in detection of food- borne pathogens [8].

The aim of the present study is to evaluate a molecular method for detection of *Salmonella* in chicken samples and compare it with the conventional method, also to determin the prevalence and serotypes of *Salmonella* spp.

Methodology

Sample collection and Salmonella detection: A total of 250 samples of whole chicken carcasses were collected, (100) from local chickens were obtained from local poultry abattoir and retail shops and (100) from imported chickens purchased from

retail shops as well as (25) liver samples from local chickens and (25) from imported chicken were also collected , during April 2018 – September 2018.

All samples were labeled , recorded and immediately transported to the microbiology laboratory, college of Veterinary Medicine, University of Duhok and microbiological analyses were carried immediately after samples collection. The pre-enrichment of collected samples were conducted according to the procedure described by Medici et al [9].

The isolation and identification of *Salmonella* was performed According to (ISO 6579:2002(E), 2002), [10]. For chicken carcases , the whole carcass was rinsed with 250 ml BPW (puffered peptone water) (HI Media, India) and 10 ml of rinsed was directly incubated at 37C for 24 hrs. While for liver samples, a piece of 25 gm was homogenized with 225 ml of BPW and incubated as previously (pre-enrichment). After that 10 ml of pre-enrichment was added to 90 ml of Rappaport-Vassiliades broth and incubated as the first step (selective enrichment) [11]. After that , 1-2 lopffuls were directly streaked quarterly onto Xylose Lysine Deoxycholate agar (XLD) and incubated at 37 °C for 18-24 hours. The suspected colonies of *Salmonella* spp.,(red with black in the center) were further sub cultured on Brolliance *Salmonella* chromogenic agar (Oxoid) with previous incubation conditions. Then Magenta colored colonies from this chromogenic agar were further confirmed by standard biochemical tests; triple sugar iron agar (TSI), citrate utilization test, urease test [12,13,14].

DNA extraction: The DNA was extracted from biochemically identified *Salmonella* isolated through boiling method which was previously described [15]. Briefly, 4-5 colonies from XLD agar has been taken and added to small tubes containing 200 µl of sterile distilled water. The tubes were mixed through vortexing and incubated at 99°C for 20 mints on heating block , the sample was directly cooled at on ice for 5 min. before it was centrifuged at 15,000 ×g for 10 min. The supernatant has been taken for PCR amplification.

PCR amplification: Amplification was carried out using primer pairs that were previously tested [16]. Forward primer; 16SF1 (5′-TGTTGTGGTTAATAACC GCA-3′) and reverse primer; 16SIII (5′-CACAAATC CATCTCTGGA-3′) for amplification

of 16S rRNA gene (Humanizing Genomic Macrogen) the expected amplicon size was 572 bp. The polymerase chain reaction PCR was performed using 50 µl reaction volumes containing; 25 µl Prime Taq premix 2X Master Mix (GeNet Bio), 15 µl of nuclease free water and 2.5 µl of each primer as well as 2.5 µl of the bacterial DNA extract. The amplification conditions for 35 cycles were composed of an initial denaturation at 95 °C for 5 min,then 35 cycles of a denaturation at 95 °C for 2 min , annealing at 55 °C for 30 s and primer extension at 72 ° C for 1 min and final extension at 72 °C for 10 min. The PCR products were visualized on 2% agarose gel by using transilluminator [16].

Serotyping: The serotyping of the isolates identified as *Salmonella* was carried out in the central public health Laboratory, Baghdad / Iraq. After final confirmation by biochemical tests, appositive isolates with one to TSI slant then incubated at 37 C for 24 hrs. After the appearance of slants results, they were submitted on ice for salmonella serotyping center in Baghdad, Ministry of health for complete serotyping of isolates.

RESULTS

A total Twenty one 21(8.4%) isolates of *Salmonella* spp. were obtained, by conventional methods,while out of (21) isolates only 12 isolates were confirmed by PCR (Figure 1). The highest prevalence of *salmonella* isolates were detected in local chicken carcasses 12 (12%) followed by imported chicken carcasses 8 (8%) and local chicken liver 1 (4%) by conventional methods. On the other hand , the highest prevalence of isolates were seen in local chicken carcasses 9 (9%), imported chicken carcasses 2 (2%), local chicken liver 1 (4%) by the molecular technique. In addition to that , a total of 12 *Salmonella* serotypes were identified by serotyping technique including 11*S. enteritidis* and only one *S. Ohio*(Table. 2).

Additionally, the PCR products of the positive isolates were sequenced for further confirmation.

Table 1: Prevalence of Salmonella isolates in chickens and liver samples

Samples	Samples No.	Ve+ samples(%) Conventional methods	Ve+ samples(%) Molecular methods
Local chicken carcasses	100	12 (12%)	9 (9%)
Imported chicken carcasses	100	8 (8%)	2 (2%)
Local liver	25	1(4%)	1 (4%)
Imported liver	25	0	0 (0%)
Total samples	250	21 (8.4%)	12 (4.8 %)

Table 2:Distribution of non-typhoidal salmonella serotypes among the total PCR confirmed isolates

Serotypes locations	Salmonella enteritidis	Salmonella ohio
Imported chicken carcass	2	0
Local chicken carcass	8	1
Local chicken liver	1	0
imported chicken liver	0	0
Total	11	1

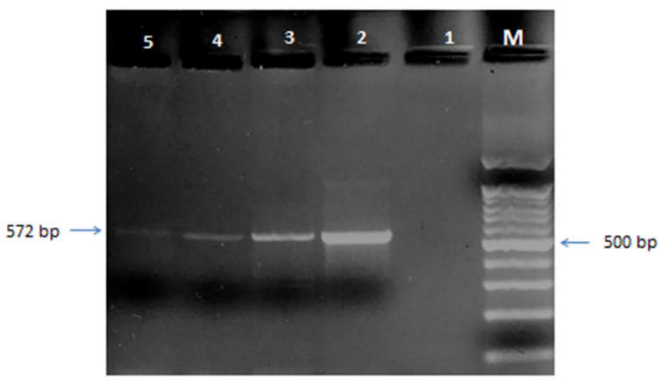

Figure 1:Gel electrophoresis of Salmonella 16S rRNA gene specific PCR products (indicated by arrow). Lane M: 100 bp marker. Lane 1 the negative and 2 positive controls, respectively Lane 3, Salmonella from local chickens; lane 4: isolates from imported chicken

DISCUSSION

The outbreak of *Salmonella* infections has been increasingly repeated, some times comes in a deadly form. The majority of the outbreaks are due to the ingestion of the contaminated animal products with *Salmonella* [17]. The continuous monitoring of this infection is a crucial for the public health program as contaminated food with *Salmonella* species might cause health issues[17].

In this study, 250 samples were examined for the detection of *Salmonella* either by conventional culture methods and PCR. The conventional methods showed that a total of 21 (8.4%) were positive for *Salmonella* ; local chicken carcasses were12 (12%) and imported chicken carcasses were 8 (8%) inaddition to local chicken livers were 1 (4%).

The conventional culture methods consider, non-selective and selective enrichments as well as culturing on selective and differential agars followed by biochemically and serologically confirmations , that could take upto 7 days to get results. Inaddition , PCR was performed on all positive samples by conventional methods as PCR is considered either as more specific and more sensitive than conventional methods.

PCR results showed that the positive samples of conventional methods have reduced to 12 (4.8%) in a way that local chicken carcasses were 9 (9%), imported chicken carcasses were 2 (2%)and local chicken livers were 1 (4%). These results showed that conventional results require further validation by molecular methods to get more accurate results. Some studies have shown that using PCR for *Salmonella* detection in food, especially for a chicken is more sensitive and reliable than the conventional culture methods [18,19].

Other studies have revealed that clinical samples contain inhibitory agents that would interfere with the PCR reaction , therefore they have proposed the use of a general internal amplification controls in order to avoid false negative results [20,21]. In this study, pure isolated *Salmonella* colonies were used for further confirmation by PCR

to avoid the presence of inhibiting agents effect on amplification efficiency. Finally, our results revealed that conventional culture methods for *Salmonella* identification are not adequate for confirmation therefore PCR is needed for more identification as it is more sensitive and more specific.

The findings showed a high occurrence (9% and 1%) of *Salmonella spp.*in chicken carcases and liver of local markets respectively, while a lower detection (2% and 0%) in the imported carcases and liver respectively. The serotyping results showed that only one local Chichen carccas was *Salmonella ohio* and all the other 11 positive samples were *Salmonella enteritidis*. These findings indicate a low level of hygiene and sanitation measures taken by local slaughtering process.

In conclusion contamination of chicken meat and liver with *Salmonella* indicate bad microbiological quality of retail chicken, which may due to contamination occur during processing or distribution. This study indicates the requirement of considering a serious hygienic measure in the slaughtering process of poultry in order to reduce the potential contamination of the carcasses and liver. As chicken processing requires many steps, there are proofs that some of the processing stages ease the contamination of chicken carcasses. Furthermore, continuous monitoring studies are required in order to evaluate the rate of contamination as well as the serotypes and serovars involved in the contamination of the poultry products.

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Conflict of interest statement

We declare that we have no conflict of interest.

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عزل وتشخيص السالمونيلا من عينات الدجاج بالطرق التقليدية والتقنية الجزيئية

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الخلاصة

الهدف من هذه الدراسة هو تقييم الطرق الجزيئية للكشف عن السالمونيلا في عينات الدجاج ومقارنتها بالطرق التقليدية. تم جمع ما مجموعه ٢٥٠ جثة كاملة من الدجاج. ١٠٠ دجاجة محلية ، ١٠٠ دجاجة مستوردة ، ٢٥ كبد دجاج محلي و ٢٥ كبدًا مستوردًا. حيث تم عزل ٢١ (٨.٤ ٪) عزلة من أصل ٢٥٠ عينة بالطريقة التقليدية، وتم عزل١٢ (١٢%) من الدجاج المحلية، ٨(٨%) عزلة من الدجاج المستوردة، و ١ (٤%) من كبد الدجاج المحلي. اما بطريقة التقنية الجزئية تم عزل ١٢ عزلة للسالمونيلا .تم اكتشاف أعلى نسبة انتشار للسالمونيلا من ذبائح الدجاج المحلية ١٢ (١٢ ٪) تليها ذبائح الدجاج المستوردة ٨ (٨ ٪) وكبد الدجاج المحلي ١ (٤ ٪) بالطرق التقليدية. من ناحية أخرى ، انخفضت النسبة إلى ٩ (٩٪) في الدجاج المحلي ، ٢ (٢٪) في الدجاج المستورد ، ١ (٤٪) في كبد الدجاج المحلي بواسطة PCR. تم عزل المصلب في مختبر الصحة العامة المركزي. علاوة على ذلك ، تم تحديد ما مجموعه ١٢ من أنماط السالمونيلا المصلية من خلال أسلوب التنميط الموضعي بما في ذلك ١١ S. enteritidis و واحدS. ohio . أخيرًا ، كشفت نتائجنا أن الطرق التقليدية لتحديد السالمونيلا ليست كافية للتأكيد ، لذلك يلزم أن يكون PCR أكثر تحديدًا لأنه أكثر حساسية وأكثر تحديدًا. تشير هذه الدراسة إلى حاجتها إلى تدبير صحي صارم في عملية لحوم الدواجن للحد من التلوث المحتمل للمنتجات. علاوة على ذلك ، هناك حاجة إلى دراسات مراقبة مستمرة لتقييم مدى انتشار ومعدل التلوث وكذلك طبيعة مسببات الأمراض المشاركة في التلوث.

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