IDENTIFICATION AND SEROTYPING OF *SALMONELLA* ISOLATES ISOLATED FROM SOME ANIMAL MEAT

Alaa A. Ahmed, Mohammed H. Khudor

Department of Veterinary Microbiology and Parasitology , College of Veterinary Medicine,

University of Basrah, Basrah,raq.

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Corresponding Author: mohamedh_79@yahoo.com

ABSTRACT

This research was already prescribed for the identification and serotyping of *Salmonella* isolates from 205 samples totally different, including frozen chicken meat(thigh, wings, liver, 40 samples for each one), 40 samples of frozen beef meat 4 and fresh beef meat (liver, muscle, and ground beef , 15 each) in Basrah throughout the amount between 4th October 2017 to 27th February2018. Results showed that the overall rate of Salmonella isolates were 22.4 percent by conventional victimization isolation of Xylose Lysine Deoxycholate Agar (XLD).The highest percentagewas appeared in liver of imported frozen chicken meat (80%), while the lowest percentage was in liver of local fresh beef (6.6%). The results of identification of *Salmonella* by conventional biochemical test and API 20 E system were76.0% and 84% , respectively, while the results of PCR technique by using 16srRNA was(20/20)100% . The result of serotyping on *Salmonella*isolates revealed that serotype *Salmonella*Typhimurium (40%), whereas the lower number was *Salmonella* serotype Kentucky (15%).

INTODUCTION

The *Salmonella* species can be a cluster of gram - negative bacterial happiness for the Enterobacteriaceae family, which causes disease in the humans worldwide. Of this genus, 2 species are recognized; S. Enterica and S. Bnongori and 2,600 serotypes are delineated these days.Significantly, about 93.8 million sicknesses, of that eightythree million are foodborne and 115,000 deaths annually are caused by non - typhoidal

salmonella (1), where uncooked chicken meat has been identified as a major source of human food poisoning (2, 3).

Salmonella serotypes remain a possible threat to the public and environmental health.*Salmonella* infection may not lead to fatal disease, but rather it will remain located within the intestinal tract leading to gastroenteritis or may have a blood poisoning effect on many organ systems. (4,5).

Meat and chicken products are identified because 40 percent of the clinical cases related to the usage of eggs and chicken products are the main sources of transmission of enteric species to humans. (6, 7, 8)

Chickens, turkeys, cows and pigs are still the most prevalent reservoirs of animals. Different domestic and wild animals conjointly harbor these organisms. *Salmonella*entericaserovarEnteritidis could be a major explanation for food borne diseases and through last decade it's been isolated worldwide in increasing numbers. Moreover*S. enterica*serovarTyphimurium is that the most often isolated serotype worldwide. Dairy merchandise, vegetables, fruits, shellfish, beef, pork, poultry and eggs are the foremost common sources of human food poisoning (9). enteric bacteria spp. were a typical food born microorganism for poultry and meat (10,11).

Salmonella can even be donated in processing facilities and in further processing plants, where cross - contamination between the carcasses can occur through direct contact with fecal matter or through the sharing of materials used in the process (12).The aim of this study was to detect the presence of *Salmonella* and *Salmonella* serotypes in local and imported meats.

MAREIAL AND METHODS

A total number of 205 samples were taken from frozen chicken meat samples (thigh, wings, liver, 40 samples for each one), frozen beef meat 40 samples and fresh beef meat (liver, muscle, and ground meat , 15 for each) in Basrahcity during the period between 4 October 2017 to 27 February2018.All samples were unbroken in sterile plastic luggage containing ice pack and transported to the Medicine lab of Veterinary biological science at the Basra University.About twenty five g of every sample was withdraw tiny items victimization sterile extractor and scissors and homogenized in sterile flask containing 225 milliliter of buffered organic compound water as a pre - enrichment broth and incubated for twenty - four hours at 37 ° C. During incubation,

1 metric capacity unit of pre - enrichment culture was transferred into sterile tubes containing 9 ml of selenite F broth, and tubes were then incubated at 37 ° C for 24 hours.A loop of each incubated tube was subsequently polite on Xylose Lysine Deoxycholate (XLD) agar and incubated at 37 ° C for 24 hours (13).

Identification of *Salmonella* isolates was done by API 20 E(Biomerieux, France) according to(14) and confirmed with *16srRNA* (15).

The serological identification of Salmonella isolates was carried out in accordance with the Kauffmann - White system [16].Serological identification of isolates were done in the Department of Microbiology, Central Public Health laboratory in Baghdad

Genomic DNA extraction

Bacterial deoxyribonucleic acid was extracted consistent with manufacture of microorganism extraction kit (Genaid,Korea). All enterics isolates had been big in five metric capacity unit ofLuria-Bertanibroth over night at 37 °C (12).The primers for *16SrRNA* to conformity identification of *Salmonella* isolates was used according to Hellberge*et al.*,(17).

F- TGT TGT GGT TAA TAA CCG CA

R- CAC AAA TCC ATC TCT GGA

The PCR was carried out in a total volume of 20 µl containing 5 µl genomic DNA from Salmonella isolates, a master mix, 1µl of each *16srRNA* - specific primers and 13 µl of deionized distilled water (DDW).The cycle conditions for the amplification of the *16srRNA* (574bp) were as follows: The initial denaturation was 95 ° C for 3 minutes, followed by 30 cycles consisting of 30 seconds at 95 ° C, 30 seconds at 54.1 ° C, and the final extension of 1 minute at 72 ° C (15).

RESULTS

The study findings discovered that the occurrence of enteric bacteria in chicken and beef samples was 46/205 (22.4 %) on Xylose Lysine Dexycholate Agar (XLD).The highest percentagewas appeared in liver of imported frozen chicken meat 16/20 (80%), while the lowest percentage was in liver of local fresh meat 1/15 (6.6%) table 1.

The outcomes *Salmonella* isolate identification using conventional biochemical test and API 20 E system were 35/46 (76 %) and 12/25 (84 %) respectively.

For further conferment twenty of *Salmonella* isolates that were known by API 20E system were subjected to deoxyribonucleic acid extraction and PCR assay by amplification of fragment of *16srRNA* (574bp).Twenty (100 %) isolated PCR tests showed positive results(figure 1).The results of 20 serotyped *Salmonella* isolates showed that serotype*Salmonella*Typhimuriumwere8 (40%),serotype *Salmonella* Enteritidis 5 (25%), serotype *Salmonella* Munchen 4 (20%) and serotype *Salmonella* Kentucky 3(15%) ,table (2).

Table (1): Percentage of *Salmonella* isolates according to XLD,SS and MacConky agar

Sample	Type of sample	No.of sample	No.of positive on XLD	Positive %	No.of positive on SS	Positive %	No.of positive on MacConky	Positive %
Local frozen chicken meat	Thigh	20	5	25	4	20	8	40
	Wing	20	5	25	7	35	7	35
	Liver	20	2	10	1	5	2	10
Total		60	12	20	12	20	17	28.3
Imported frozen chicken meat	Thigh	20	7	35	4	20	4	20
	Wing	20	3	15	3	15	5	25
	Liver	20	16	80	19	95	19	95
Total		60	26	43.3	26	43.3	28	46.6
Local fresh beef meat	Muscle	15	2	13.3	15	100	10	66.6
	Liver	15	1	6.6	12	80	13	86.6
	Ground meat	15	2	13.3	10	66.6	12	80
Totl		45	5	11.11	37	82.2	35	77.7
Imported frozen beef meat	Muscle	40	3	7.5	25	62.5	15	37.5

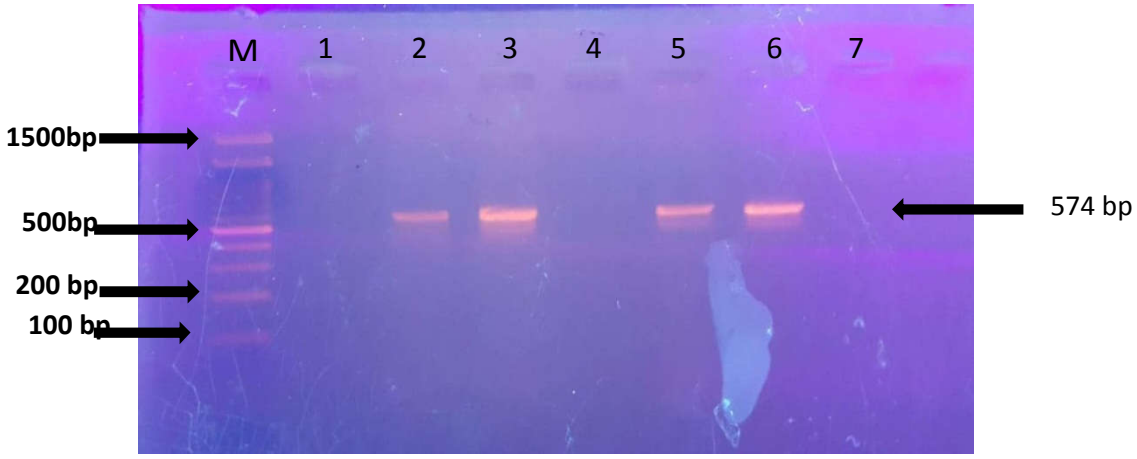

Figure (1) PCR amplification of *16srRNA* (approximately 574pb) for conformity of *Salmonella* isolates in 1.5% agarose gel stained with ethidium bromide.Lanes:M, Marker. 1, 4 negative, 2,3,5,6 ;positive band for 16srRNA gens,7 control negative

Table (2) :Salmonella isolate serotypes and their percentages

Serotype	Number	Percentage
Salmonella Typhimurium	8	40%
Salmonella Enteritidis	5	25%
Salmonella Kentucky	3	15%
Salmonella Munchen	4	20%

DISCUSSION

The results of bacterial cultures obtained in this study showed that the total range of Salmonella isolates isolated from frozen chicken (local and imported) were38/120 (31.6%) These results were almost similar to the findings of (18) and (19), which discovered that Salmonella isolates were present in general (24.7%)and(27%) respectively and disagree with Todd (20), (21), (22) who found that the overall presence of *Salmonella* were 13.3%,15.4% and 10%, respectively. These differences could be due to the impact of many factors such as the DE feathering method, which could develop the microorganisms between the carcasses or from the DE feathering instrumentation depending on an increase in the number of psychrotrophs and

mesophiles on the carcasses.The evisceration method offers the possibility of cross - contamination from the hands of people, instruments and workers (23).

The total number of *Salmonella* isolated from local fresh beef meat were 5/45 (11.11%). These results wereagreed with (22) and (24) who found that overall presence of *Salmonella* in fresh meat product was 13.3%, 10.66% respectively, whereas the results were disagreed with (25),(26) and (27) who found that the overall presence of *Salmonella* in fresh meat product was 8.5%, 1.33%, and 4.2%,responsibly. The high prevalence of salmonella in meat goods may indicate meat contamination during trimming or processing, as well as spoilage from blenders, air, packaging materials and staff hands.(28) The total number of *Salmonella* isolated from imported frozen beef meat was 3/40(7.5%). These results are in agreement with(24) and (29) who showed that the overall percentage of *Salmonella* spp. was 6.6% and 8.5% respectively, whereas the study results disagreed with (30,31, 32) who found that the percentages were 3.4%, 12%, and 16.9%, respectively. This difference in the results may be due to contamination of the meat equipment and workers hand. In the gift study, thePCR assay that was used for the identification of *Salmonella* isolates depend on *16srRNA* gene showed that 16S ribosomal RNA gene is more accurate for identification and specificity and also for detection and discrimination between species of *Salmonella* and non - *Salmonella*(33). The results showed that (100%) ofisolates were demonstrated bands approximately 574 bp which wasconfirmedthe presence of *16SrRNA* gene. These results are in agreement with (34) who found 92% in white meat but the results disagree with (15) who found 63%in white and red meat. ARecent study on the molecular medical specialty and in vitro antimicrobial status of *Salmonella* isolated from poultry in geographical area depression, Asian country showed that everyone the isolates of *Salmonella* were tested by genus-specific enzyme chain reaction (PCR), victimization the 16S ribosomal ribonucleic acid (rRNA) primers (35).

A total of twenty isolates from poultry and beef wereserotyped,the result was 40% serotype*Salmonella* Typhimurium, 25% serotype*Salmonella* Enteritidis, serotype *Salmonella* Munchen 20%and 15% serotype*Salmonella* Kentucky .According to the results of serotyping, the percentage of serotype *Salmonella* Typhimuriumfrom thetotal *Salmonella* serotypes in current study was40%.These results are in agreement with (36), (37) who found that percentage of serotype *Salmonella* Typhimuriumwas 44% and 40.35%, respectively, whereas the current results disagree with other

researchers like (38), (39) and (40) who found that the total percentages of serotype*Salmonella* Typhimurium were 23.55%, 2.5% and 8%, respectively.In addition in the current study serotype*Salmonella* Enteritidis serotype percentage was 25%, these results are corresponded with (41) who found 20%. These in contrast the results disagree with the othersthat were obtained by (42), Eldesouky (25) and(43) who found total percentage of serotype *Salmonella* Enteritidis were 38.4%, 47% and 68%,respectively.Moreover the result of *Salmonella* Munchen serotype obtained in this study was 20%and *Salmonella* Kentucky serotype was observed in 15%.These results are in agreement with (44) who found that the percentages *Salmonella* Kentucky serotype was 17%, while they are disagreed with(45) who found 6.25% only.

تشخيص وتحديد الأنماط المصليه لعزلات السالمونيلا المعزولة من بعض لحوم الحيوانات

الاء عبد الهادي احمد، محمد حسن خضر

فرع الاحياء المجهرية، كلية الطب البيطري ، جامعة البصرة ، العراق

الخلاصة

أجريت هذه الدراسة للتعرف على أنماط السالمونيلا المعزوله من لحومبعض الحيوانات.جمعت ٢٠٥ عينة مختلفة شملت لحم الدجاج المجمد (الفخذ ، الأجنحة ، الكبد ، ٤٠ عينة لكل منها) ، لحم البقر المجمد ٤٠ عينة ولحم البقر الطازج (الكبد ، العضلات واللحم المفروم ، ١٥ لكل منها) من الاسواق المحليه في البصرة خلال الفترة ما بين ٤ أكتوبر٢٠١٧ إلى ٢٧ فبراير ٢٠١٨. أوضحت النتائج أن نسبة عزل السالمونيلا كانت ٢٢.٤٪ باستخدام العزل على وسطXylose Lysine Deoxycholate (XLD)وظهرت أعلى نسبة في كبد لحم الدجاج المجمد المستورد (٨٠٪)، بينما كانت أقل نسبة في الكبد من اللحوم الطازجة المحلية (6.6٪). تم التعرف على عزلات السالمونيلابواسطة الاختبار البيوكيميائي وبواسطة استخدام نظام API 20 Eبنسبة ٧٦% و ٨٤% على التوالي .تم التاكد من عزلات السالمونيلابتقنية تفاعل البلمرة المتسلسل(PCR) باستخدام *16srRNA*.تم تحديد الانماط المصليه لعزلات السالمونيلا لنماذج اللحوم اعلاه،فكانت اعلى نسبةللنمط المصليS.Typhimurium (٤٠ ٪) ، في حين كان أقلنمط مصلي هو(S.Kentucky١٥%).

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