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DETECTION OF *SalmonellaTyhpimurium* IN IMPORTED BEEF IN LOCAL MARKETS OF AL- DIWANIYIA CITY USING PCR ASSAY

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ABSTRACT

The present study was conducted to detect *Salmonella spp.* from imported beef in local markets of AL-Diwaniya cityduring the period from December 2011 to June2012. Total of 100 imported beef samples from different origins were collected randomly from several different local markets of AL-Diwaniya city. The results of present study showed out of 100 imported beef samples examined by the selective media such as Bismuth sulphate (BS) agar and chromogenic agar (64.5%) of imported beef samples were positive for *Salmonella* spp. andout of 31 isolates examined by the chromogenic agar only 24 (77.4%) isolates were positive for *Salmonella* respectively. The chromogenic agar had significantly ($p<0.05$) the highest sensitivity for frombismuth sulphate agar.The results of Api20-E system revealed that 21 out of 22 beef isolates (95.4%) were positive for *salmonella* .The Api20-Esystem had higher sensitivity for the identification of *salmonella* than the conventional biochemical tests. Single plexPCR technique has been used to confirm the diagnosis of salmonella isolates from beef. out of 21 beef isolates examined by using the primer *16s rRAN*gene only 15 (70.4%)isolates were positive for *salmonella* . out of 15 beef isolates examined by the primer *invAgene*only 6 (40%) isolates were positive for *S. Tyhpimurium* .the highest significant ($p<0.05$)prevalence of *S. Tyhpimurium* contamination were found to be 66.6%in India beef samples.The PCR results revealed that there was a significant ($p>0.05$) differences in the percent of *Salmonella* isolation between the five different origins of imported beef.

INTRODUCTION

Salmonellosis is a serious public health problem worldwide.It is estimated that approximately 70%–80% of food borne bacterial outbreaks were caused by *Salmonella* in some country like China (1). And In the United States Salmonella infections (approximately 32,000 annually) were reported during 1998–2002 (2). , beef and poultry /chicken meat have been recognized as significant sources of human salmonellosis (3).*Salmonella* serotypes, *S.Typhimurium* is one of the most important agents of food borne Salmonellosis in humans (4).It was estimated that approximately 75% of human salmonellosis cases were due to contaminated food products, such as beef, pork, poultry and Chicken products(5) In Germany, from 2001 to 2005, in Italy, an outbreak of *S. Typhimurium* phage involving 63 cases was reported, the aim of this study was to isolated and identification *salmonella spp.* and S*almonella Typhimuirim* from beef imported in the market of Al-Diwaniyacity.

MATERIALS AND METHODS

Sample collection

Atotal of 100 beef sampleswere collected from different market in Al-Diwania city with different origin include different trademark , about 25 gfrom sample were placed in enrichment broth tetrathionatethan transported laboratory/the unit of zoonotic diseases researches the college of veterinary medicine then incubate for 18-24 h at 37°C

Isolation and identification of*Salmonella* spp.:-

The samples were cultivated on to selective media such asBismuth sulphate (BS) agar andchromogenicagarfor identification of *Salmonella* colonies and then samples were subjected to biochemical tests to confirm by using Api20-E system, Colonies that showedbiochemical characteristics similar to that of *Salmonella* spp. The confirmation was identified by PCR assay with *16s rRNA*and*invA*genesfor the detection of*Salmonellaspp*

Specific primers sequence used for PCR amplification:

The primers used for the detection specific sequence of(16s*rRNA)*[**6],**and *invA* gene **[7]**.These primers are specific for designed in this study by using NCBI Gene Bank and Primer: online and provided by (Bioneer company, Korea) as following Table (1):

Table (1): Specific primers used for the detection of *16srRNA gene* and *invA gene*

Sequence	Orientation	Position	Size of PCR product(bp)
CGG.,ACG,GGT,GAG,TAA,TGT,CT	Forward	*16s rRNA*	406
GTT,AGC,CGG,TGC,TTC,TTC,.TG	Reverse		
ATG,CCC,GGT,AAA,CAG.ATG,ATG,AG	Forward	*invA*	558
CTC,GCC,TTT,GTC,GGT,TTT,AG	Reverse		

Genomic DNA extraction

The extraction of DNAfrom *Salmonella*isolates was performed according to Genomic DNA kit provided by gene aid company (USA). The amplifiedof DNA products were analyzed with electrophoresis on 1% agarose gels stained with ethidium bromide and visualized by UV illumination depending on DNA marker (2000 bp DNA ladder).

Preparation of PCR reactions

ThePCR amplification mixture (20µl) whichwas used for the detection each geneincludes 5 µl of (PCR PreMix Lyophilized**)**, which provided by Bioneer (Korea.)include: bacterially derived TaqDNA 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. µlpcr water to complete the amplification mixture to 20 µl. The PCR tubes containing an amplification mixture were transferred to*thermocycler* and started the program foramplication of the *16s rRNA andinvAgenes.*30 cycles of PCR, with 1initial denteration 1cycle 95C˚ for 1 min.then 5 min at 95C˚ (denaturation), 30 s at 55 C˚ (annealing),and 45s at 72 C˚ (extension). And 1 cycle for 7 min at 72c˚ (final extension).

RESULTS

In this study, *Salmonella*sppwasisolatedby use bismuth sulphate agar with total percentage was 64.5 %(31/48).Where, the highest ratio of isolation was from beef (India origin) .while, the percentage of isolation on chromogenic agar was 77.4% (24/31) table (2).The colonies of

Salmonella spp.on chromogenic agar were variable in size convex and mauve in color (Figure1)

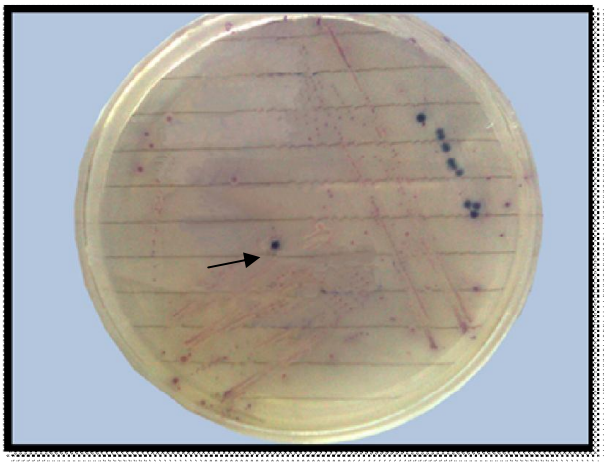

Figure (1) Colonies of *Salmonella* spp. on chromogenic *Salmonella* agar

Table (2): The percent of *Salmonella* spp. isolates by using Cutler media

Beef samples	Cutler media					
	Bismuth sulphate agar			Chromogenic agar		
Origin	No. of Sample	No. of positive	%	No. of Sample	No. of positive	%
India	11	8	72.7^{A}	8	6	75 ^{A}
Brazil	10	7	70^{A}	7	5	71.4^{A}
Australia	9	4	44.4^{B}	4	4	100^{B}
A.U.D	8	6	75^{C}	6	5	83.3^{C}
India	10	6	70^{A}	6	4	66.6^{D}
Total	48	31	64.5^{b}	31	24	77.4^{c}

-Different capital letters in a column reveled significant differences ($p < 0.05$) between the percent of isolation

- Different small letters in a rows reveled significant differences ($p < 0.05$) between the biochemical tests.

According to the reading of Mini API 20E system that showed 21 isolatespositive from 24samplewith percentage 92% .While The confirmed diagnosis of *Salmonella*spp. were performed by using single plex PCR to detect *16s rRNA gene* the percentage was 68.1%(15/21) and the high ratio for isolation *16s rRNA gene* from beef(India origin) was66.6% while ,the lower ratio was from beef(Brazil origin)(table 3), (Figure 2).The percentage for detect *in vAgene* for*S.Typyimuirim*serotype was 40 %(6/15).The highest rate forisolation of*S.Typyimuirim*was 100% from beef (Brazil origin)while, the lower was inbeef samples from (Australia andA.U.D origins)(Figure 3).

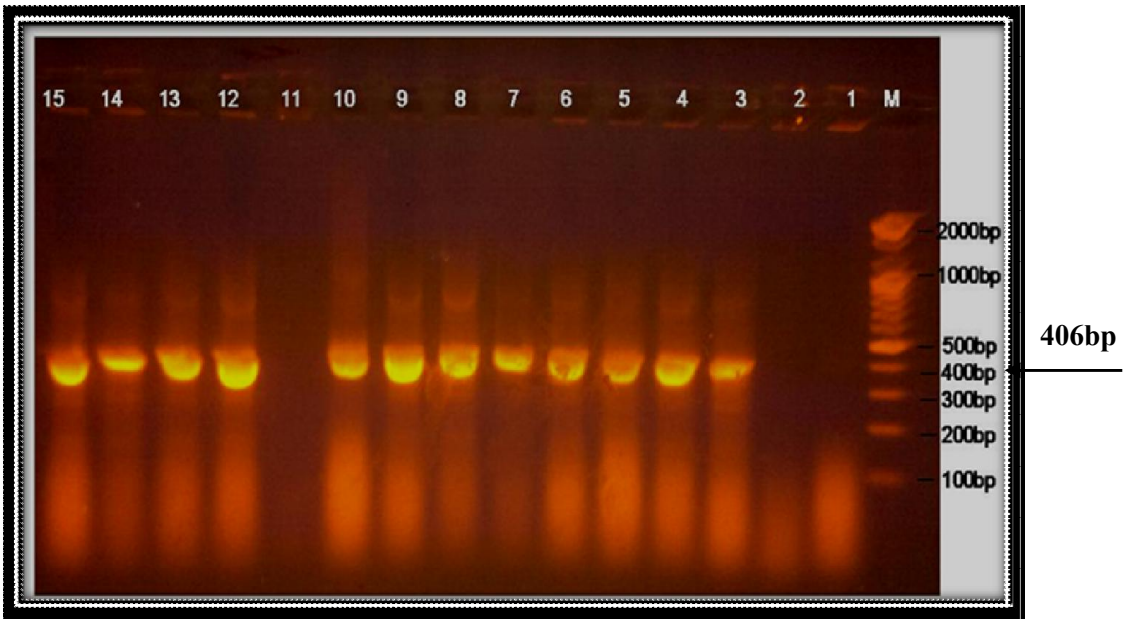

Figure 2:DNA amplification of a 406 bp of Salmonella spp.detecting *16s r RNA* gene using singleplex PCR lane 11 negative control, lane 2,1 negative results ,lane 3,4,5,6,7,8,9,10,12,13,14,15 positive results as *Salmonella spp*. Lane M 2000bp marker (ladder).

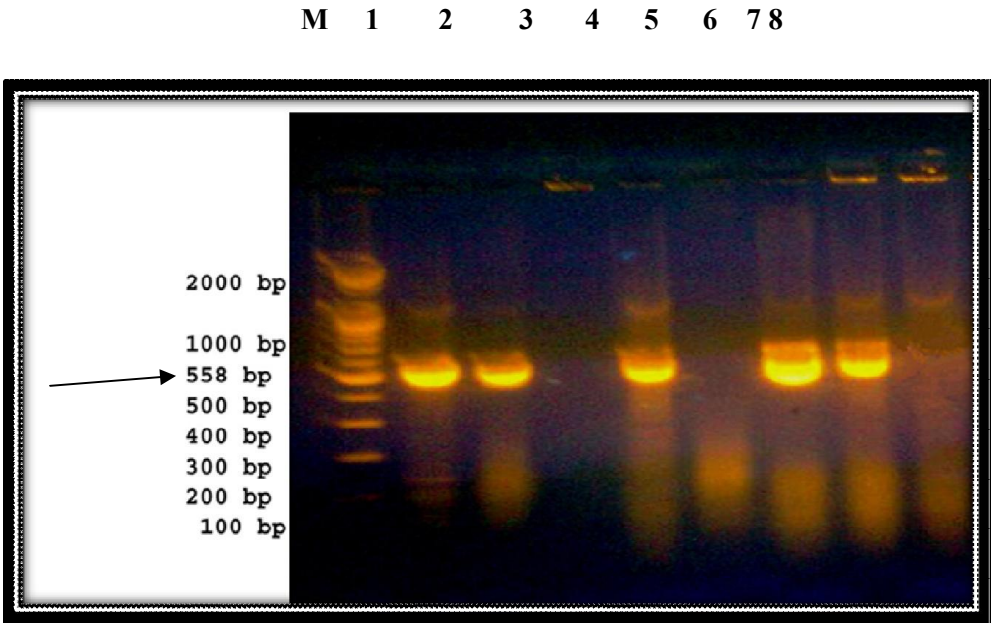

Figure 3 :DNA amplification of a 558 bp of salmonella spp .detecting *invA* gene using singleplex PCR lane 3conterol results ,lane,1, 2,4,6,7,positive results as *S. Typhimuirim* spp. Lane 5,8negative result , lane M 2000bp marker (ladder).

Table 3:- Detection of *Salmonella* sp. and *Salmonella. Typhimuirim* by single plex PCR

Beef Sample	*16r RNAs gene*			*invA* gene		
Origin	No. tested sample	No. of positive	%	No. tested sample	No. of positive	%
India	4	3	75^{A}	3	2	66.6^{A}
Brazil	4	2	50^{B}	2	0	0^{B}
Australia	4	4	100^{C}	4	1	25^{C}
A.U.D	5	2	40^{D}	2	1	50^{D}
India	4	4	100^{C}	4	2	50^{D}
Total	21	15	71.4^{a}	15	6	40^{b}

-Different capital letters in a column reveled significant differences ($p < 0.05$) between the percent of isolation - Different small letters in a rows reveled significant differences ($p < 0.05$) between *16s RNA* gene &*invA* gene.

DISCUSSION

Common sources for transmitting these food borne pathogens are raw meat including beef and beef product. *Salmonella* was a primary cause of bacterial gastroenteritis and responsible for 1.4 million case of human illness which was approximately 30% of all reported cases of food poisoning in the united state **(8).** In the present study the prevalence of *Salmonella* spp.based in Bismuth sulphate agar was 64.5%.This result also came approval for studies conducted within the country by **(9)**who isolated salmonella from imported beef when use bismuth agar . As well as agreed**,(10)** who wereisolated *Salmonella* from beef in Malaysia was 72.2%on other hand our percentage higher than that reported by (**11)** who reported 12% (30 /250)samples of beefmeat.Several bacteriological selective media have been used to isolated *Salmonella spp*. like 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**), In this study, the Chromogenic media were more efficient and presented,(13) who reported 79.%3 (23/29)samples of ground beef were contaminated with*Salmonella*.The chromogenic agar had significantly ($p>0.05$)the highest sensitivity forthe identification of*Salmonella* from samples in comparison to both the bismuth*Salmonella spp*. 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 percent of isolation *Salmonella spp*. according to the reading of results of API 20-E system were 95.4%.The Api20-Esystem had higher sensitivity for the identification of Salmonella than the conventional biochemical tests.In this work molecular genetics study has been carried out to identify the genetic characters of Salmonella by using of *16s r RNA gene or invA gene* specific PCR **(15)** .The percentage of isolation of *Salmonella* frombeef by using single plexPCR technique was71.4 %(15/21) this percent was closer to results reported by **(16)** who obtained 62%. In this study, the percentage ofconfirmation of *S .Typhimurium* was 40 %(6/15). The resultswere higher than **(17,18),**when using PCR to detected salmonella *spp.* in beef sample the PCR results revealed that was a significant differencesin($p>0.05$)in the percent of salmonella isolates between the different origins of importedbeef and chicken meats. The final result of isolation 15%(15/100) this percentage refer to a highly contamination with salmonellain importedbeefin Iraq. The conclusion, Un

hygienic practices and poor sanitation technique in the slaughter houses ,transportation vehicles, butcher stores and handlers that may introduce such salmonella organism in meat were reflected on the highest prevalence of contamination of red and white meats with such organism . Consumer educational efforts are needed for proper cooking process of red meat before consumption with improving personal sanitation techniques in the line of meat preparations to ensure the safety of meat and meat products for human consumption.

تشخيص جراثيم السالمونيلا تايفيميوريم في لحوم الابقار المستوردة في الاسواق المحلية لمدينة الديوانية باستخدام تقنية سلسلة تفاعلات البلمرة

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الخلاصة

اجريت هذه الدراسة لغرض الكشف عن جراثيم السالمونيلا في لحوم البقر المستورده في الاسواق المحلية لمدينة الديوانية وللفتره من كانون الاول 2011ولغاية حزيران 2012. تم جمع 100 عينة من لحوم البقر من مناشيء مختلفة عشوائيا وكانت نسبة العزل على وسط سلفات البزموث بمقدار 64.5 %(48/31) وقد اعطت عزلات السالمونيلا على هذا الوسط مستعمرات دائرية سوداء ذات لمعة معدنية وقداستخدم وسط الكروم اكار كوسط انتقائي خاص لعزل السالمونيلا وكانت نسبة العزل على هذا الوسط (31/24) وبنسبة 77.4% وكان شكل مستعمرات السالمونيلا على هذا الوسط وردية محدبة وبأحجام مختلفة وقداعطى الكروم اكار خصوصية عالية وبفرق معنوي($P < 0.05$)مقارنة مع وسط سلفات البزموث. واظهرت نتائج الاختبارات البايوكيميائية ان 22 عزلة من اصل 24 عزلة موجبة وبنسبة 96.1%. اما نظام API20-E فقد استخدم لتأكيد نتائج الاختبارات البايوكيميائية حيث اكدت النتائج الموجبة 21 عزلة من اصل 22 عزلة وبنسبة 95.4% واعطى نظام الAPI20-E حساسية عالية وبفارق معنوي ($P< 0.05$) في تشخيص جرثومة السالمونيلا مقارنة بالطرق البكتريولوجية التقليدية . وقد استخدمت تقنية Sinlgplex PCR في تشخيص النمط المصلي *S.Typhimurium* بأستخدام البادئ النيوكليوتيدي *invA*في 6 عزلات من مجموع 15 عزله وبنسبة 40% للنمط المصلي m*S.Typhimuriu*في لحوم البقر، قدبينت نسبعزل عالية وبفارق معنوي ($P< 0.05$)من النمط المصلي*S.typhimurium*وبنسبة 66.6% في المنشأ الهندي للحوم البقر.

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