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ISOLATION AND IDENTIFICATION OF *YERSINIA ENTEROCOLITICA* FROM LOCAL OVINE MEAT IN THE MIDDLE REGION OF IRAQ

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Abstract

Yersinia enterocolitica has ranked a third among the pathogens that most frequently cause gastrointestinal disorders transmitted to humans through food materials, especially contaminated meats. The meat infected *with Yersinia enterocolitica* had no change in apparent texture or smell. The aim of this research is to survey the frequency of *Y. enterocolitica* in ovine meat, compare their ratio of infection between the season ,To carry out this study (125) samples of local ovine meat were collected by random sampling from the middle region of Iraq. The samples were divided into two groups steak and mince, then many microbiological tests (culture, & staining, biochemical Tests Api 20E, Vitik 2 and species-specific PCR amplicon for 16S RNA gene) were performed. (16%) of all of the studied Ovine meats sample were contaminated with *Y. enterocolitica*. The analysis of results with chi-square tests showed that there was a significant variation in the isolation percentage between seasons, also minced ovine meat was more contaminated than steak.

Keyword **:** *Yersinia enterocolitica*, Ovine meat, CIN, Iraq.

Introduction

Yersinia enterocolitica is a member of the *Enterobacteriaceae* family, the etiological agent of yersiniosis, an important zoonotic disease that causes symptoms ranging from a mild, self-limiting diarrhea to acute mesenteric lymphadenitis (Griffin & Carneil, 2014), transmitted to humans through water and food, especially contaminated meats and dairy products (Long *et al.*, 2010) the preservation of such food materials in refrigerator provides an opportunity for their proliferation (Saman *et al.*, 2012) regarding the absence of any symptoms, eating of such foods can cause gastroenteritis and other digestive disorders in consumers, In general, human beings were contaminated with *Y. enterocolitica* bacteria through eating contaminated red meat (Belgian, 2004) and also a great deal of serological research showed that individuals who concerned with livestock and meat are more prone to be contaminated with *Y. enterocolitica* bacteria than others (Nimfa, 1999) the untouched tissues of healthy animals are free of microbial agents, but when they are slaughtered, the bacteria living in their digestive system, the environment of slaughterhouse, and the process of preparing meat resulted in contamination of meat (Farzaneh *et al.*, 2016).

Among several pathogen bacteria which have the ability of replication on meat at refrigerator temperature, *Y. enterocolitica* bacterium is of great importance particularly due to two reasons: Their ability of growth, replication & production of Enterotoxin (Nimfa, 1999) when kept meat in refrigerators without changing smell and taste of meat, and Their ability to cause serious diseases in consumers of such contaminated meat such as Gastroenteritis, Dysentery, Mesenteric Lymphadenitis, Septicemia, Erythema nodosum (EN), Arthritis, Autoimmune thyroid disorders and even death in some cases (Razavilar, 2003; Hasani and Firouzi, 2005).

Material and Method

Sampling :The Ovine meat sample were collected randomly from local market in five governorate (Bagdad, Bable, AL-najfe , Karbala and Al- dywania) The samples were divided into two groups steak and mince in (500) gram of each, All of the samples were carried to the laboratory in cold boxes.

Bacteriological culture : The minced mead were homogenized in sterile poly ethylene sac while the steak sample were cutted to small pieces with sterile knives and homogenized then inoculated on double strength power TSB-YE broth (1**:**10) dilution food standard formula) as sample (25 g) to 9 parts (225 ml) broth, then homogenized with stomacher for (3-5) minutes, then incubated at 30-35 C for 24 hours for resuscitation of stressed or sub lethally damaged cells after that were cold enriched in Irgasan-Ticarcillin-Chlorate (ITC) or phosphate-buffered saline (PBS) (at 4C° for 72-96 hr) then The cold enriched samples were subjected to alkali treatment (0.5 ml of each culture was transferred for (20)Sec. into 4,5 ml of (0.5% KOH in 0.5% NaCl, then a loobful was streaked onto MacConkey and CIN (Cefsulodin-Irgasan-Novobiocin Agar) agar plates simultaneously and incubated at 25ºC for (48)hrs. small and smooth, with the a red center and a translucent rim colony on CIN, which were motile at 22 ºC, but not at 37 ºC were stored on agar slants at 4 ºC for biochemical identification, API 20E and Vitik 2 bacterial identification system (bioMerieux) according to the manufacturer's instructions. (Johnson, 1998; ISO, 2003; Food and Drug Administrator. 2015)

Conventional PCR :

DNA extraction Bacterial DNA was extracted from fresh *Y. enterocolitica* colonies grown in the broth containing presumptive pure culture colonies of *Y. enterocolitica* by using GeneaidPresto™ Mini DNA Bacteria Kit (Taiwan) following manufacturer's instructions for DNA extraction protocol then was stored at –20°C until used.

Primer The identity of *Y. enterocolitica* isolates was confirmed species-specific PCR amplicon for 16S rRNA gene. The primers were lyophilized and dissolved in free deionized distill water (ddH2O) to get a final concentration of 100 pmol/µl as stock solution which was kept at -20 °C to prepare10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of free deionized distill water (ddH_2O) to reach a final volume 100 µl. Primer sequences, their references, product lengths, and specific

targets are listed in Table (1).The PCR conditions for the primers listed in table 2, 10 µl of PCR amplicon were analyzed by electrophoresis on a 2.5% agarose gel. Gels were visualized under a UV illuminator and photographed through gel documentation system. A 100-bp DNA ladder (Bioneer, Korea) was used as DNA molecule size marker.

Table 1: Primers used to detect the16srDNA, genes in *Y. enterocolitica*

Primer	Size in BP	Sequence (5'-3')	Reference
16srRNA	1485	5'-AGAGTTTGATCCTGGCTCAG-' 5'-GGTTACCTTGTTACGACTT-3'	Hao *et al.*, 2016(13)

Table 2: Amplification of 16srRNA gene achieved following condition

Phase	Tm(°C)	Time	No. of cycle
Initial Denaturation	94 °C	5 min	1 cycle
Denaturation	94 °C	30 sec	35 cycles
Annealing	62 °C	30 sec	
Extension-1	72 °C	1 min	1cycle
Extension-2	72 °C	1 min	

Result

In this study, a total of 125 samples of Ovine meat, including (70) steak and (55) minced ovine meats were randomly bought from five governorate from the middle region of Iraq, out of the all 125 sample there were 33 positive isolate depending on isolation on CIN and ordinary laboratory biochemical test (Uerase +, motile at 22 ºC, but not at 37 ºC, manntiol+ Indole -/+) While there were 23 and 21 positive isolate depending on Epi 20 and Vitik 2 compact system respectively, but there were only 20 positive sample (16%) for *Yersinia enterocolitica* for 16s rRNA gene as showed in table (3).

After testing and recording the results and conducting statistical tests, we obtained that there was a significant difference ($P < 0.01$) between the season, winter sample had the highest isolation percentage (26%) then spring sample (15%) and the summer sample had the lowest isolation (2.8%) as showed in table (4) and there was a significant difference ($P < 0.05$) between steak and mince meat ,the highest percentage was from mince meat (21.8%) while steak meat was(11.4%) no significant difference ($P > 0.05$) between the five governorate as recorded in table (5).

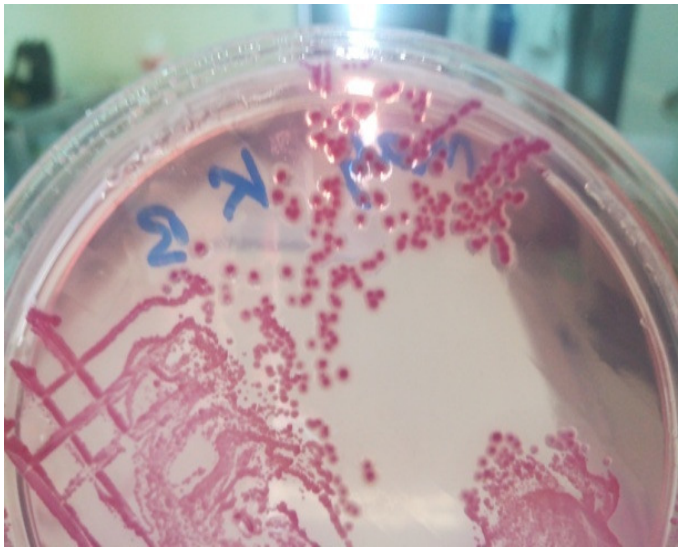

Fig. 1 : The characteristic growth of *Y .enterocolitica* isolate appeared as deep red center with a transparent margin, or "bull's-eye" on CIN medium.

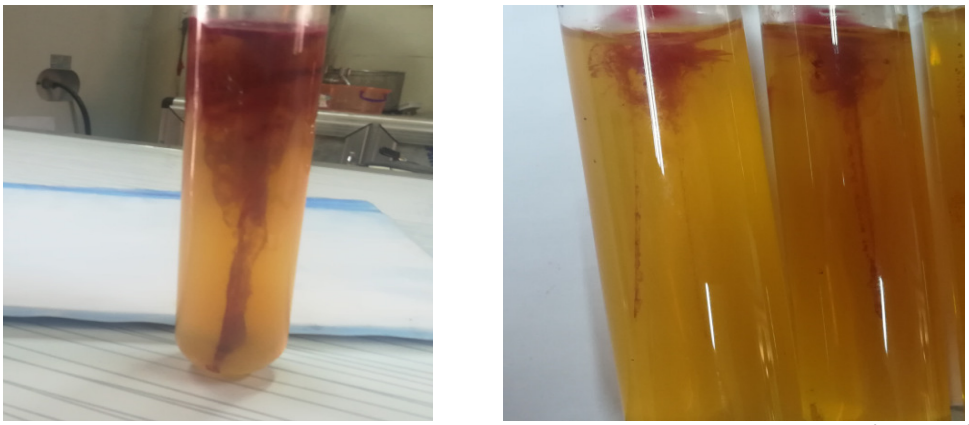

Fig. 2 : The characteristic growth figure 3 *Y. enterocolitica* on SIM *Y. enterocolitica* at 22° C at 22° C at 30° C

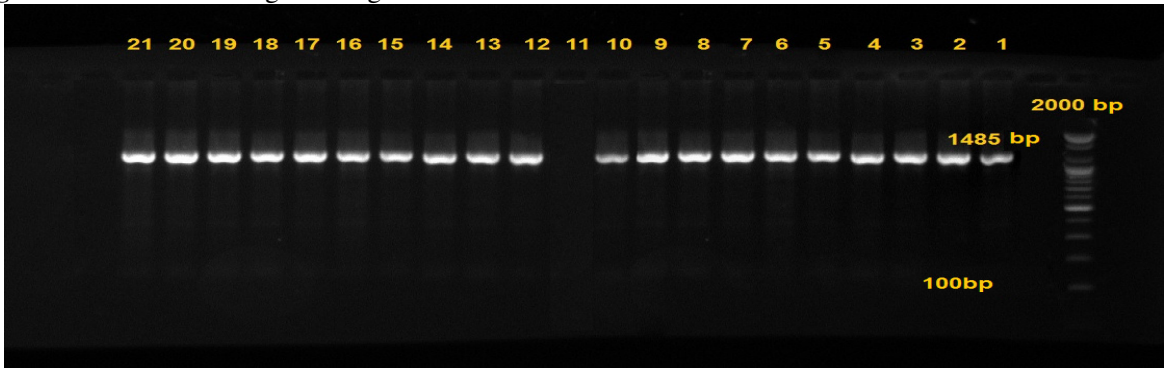

Fig. 4 : Identification species specific of 16s rRNA gene of *Y. entercolitica* PCR product at the band size 1485 bp. lane (1-21) represent gene of 16s rRNA of *Y. enterocolitica* which appear in 20 isolates, visualized under ultraviolet light (after staining).

Table 3 : Isolation of *Yersinia enterocolitica* in different tests .

Type of sample	No. samples	No. of cultuer isolate	EpI 20E	Vitek	PCR 16sgene
Local ovine meat	125	33 (26.4%)	23 (18.4%)	21 (16.8%)	20 (16%)

Table 4 : Season effect on the isolation percentage of YE from local Ovine meat

Season	Type of sample	No. of samples	No. of isolates	%.of isolates	
winter	Steak	30	6	20%	26%
	Minced	20	7	35%	
Spring	Steak	20	2	10%	15%
	Minced	20	4	20%	
Summer	Steak	20	0	0 %	2.8%
	Minced	15	1	6.6%	
Total		125	20	**16 %**	
Chi-Square (χ^2)				**17.2***	

** (P <0.01)*

Table 5 : PCR isolation percentage from steak &minced ovine meat from the governorate.

Type of sample Region	Steak sample	Positive isolate	Minced sample	Positive isolate	Total
Baghdad	20	1	15	3	4/35 11.4%
Babel	12	1	10	2	3/22 13.6%
AL-najf	12	2	10	2	4/22 18.1%
Karbala	12	2	10	2	4/22 18.1%
AL –Dywana	14	2	10	3	5/24 20.8%
Total	70	8 (**11.4%**)	55	12 (**21.8%**)	20/120 **16%**
Chi-Square (χ^2)		**4.06****			**3.75 NS**

** (P <0.05) NS non significant (P >0.05)

Discussion

Sheep were considered a source of infection with *Yersinia enterocolitica* to other species including human being (Mohammed & Ban, 2018) The bacterial isolates were obtained from 33 out of 125 examined sample in culture media, we have proven that application of single method is not sufficient for reliable identification of *Yersinia enterocolitica* because it is present a special challenge to standard biochemical test systems due to their slow growth, and high biochemical similarity (Savin *et al.*, 2012; Kinga *et al.*, 2018), However, conventional biochemical testing used in this study to confirm identification results of isolation step we used Api 20 system and VITEK 2 Compact system Which can be considered as a first-line method for identification of *Y. enterocolitica* isolates in clinical microbiology (Hans-Jörg *et al.*,1999)) The 16S RNA gene PCR amplicon still the gold standard in microbial identification (Platt-Samoraj *et al.*, 2006; Momtaz *et al.*, 2013; Alexandra *et al.*, 2015).

The results estimated that 16% (20/125) out of whole sample were contaminated with *Y. enterocolitica* depending on PCR amplicon for 16s rRNA gene and the highest isolation parentage recorded during winter These results are in consistent with the isolation of *Y. enterocolitica* from sheep recorded by (Ban & Muhammad, 2018) whom founded significantly increase in the recovery rate of *Y. enterocolitica* during the cold months (12%) as compared with temperate and hot months (spring and summer).

Results profile reflect contamination of mincemeat samples with *Y. enterocolitica* in (21.8%) which are close to the result recorded by (Ali *et al.*,2015) These findings suggest presence of complex scenarios of contamination cycle with *Y. enterocolitica*, contamination may occur at any point from animal farm to markets, shipment of stressed and infected animals, during unhygienic free selling, unknown source of mincemeat, inside abattoirs during slaughtering and evisceration, knives, butcher cutting wood surface, mincing machines, contaminated utensils, during handling and processing of meat by infected and carrier workers, flies, unclean meat environment, etc. (Siriken, 2004; EFSA, 2007). Our study applied on consumer level.

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