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Prevalence of *E. coli* O157:H7 in Raw Minced Beef at Slaughterhouses in Tripoli, Lebanon

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Abstract

Enterohemorrhagic *Escherichia coli* O157:H7 is a pathogenic strain associated with infections caused by consumption of undercooked raw meat. Sensitive and rapid detection methods for this pathogen are essential for the meat industry to ensure a safe meat supply. This study was conducted to determine the prevalence of *E. coli* O157:H7 in raw minced beef. A total of 80 raw minced meat samples were collected from eighty different slaughterhouses. Samples were investigated for the presence of *E. coli* O157:H7 by using VIDAS system followed by culture, biochemical characterization and PCR confirmation.It was observed that 18.8% (15/80) of samples were positive for *E. coli* O157:H7 by VIDAS, 33.33% (5/15) were identified as *E. coli* by conventional biochemical tests, while toxin genes amplification by PCR showed negative results for all samples 0%.

Keywords: E. coli; O157:H7

Introduction

Food-borne diseases represent a growing public health problem, both in developed and developing countries. The World Health Organization (WHO) has reported that in 2005, 1.8 million people died from diarrheal diseases worldwide. In industrialized countries, where food-borne surveillance systems have been implemented, the percentage of the population suffering from food-borne diseases each year has been reported to be up to 30% [1]. In the U.S. the Center for Disease Control and Prevention (CDC) estimates that each year, one of six Americans (48 million) get sick, 128,000 are hospitalized and 3,000 die of food-borne diseases [2]. In contrast, statistics for food-borne diseases in developing countries are difficult to estimate due to the poor or non-existent reporting systems, therefore reliable statistics on these diseases are not available thus making it difficult to estimate the magnitude of the problem however, the high prevalence of diarrheal diseases in many developing countries suggests major underlying food safety problems [1,3].

Escherichia coli is a facultative anaerobic Gram-negative, rod-shaped bacterium, belonging to the family *Enterobacteriaceae*. It is commonly found as part of the normal facultative anaerobic microflora in the intestinal tract of humans and warm-blooded animals [4]. Five virulence groups of *E. coli* are recognized, according to their effect on certain cell cultures, serological groupings, and to the disease syndromes and characteristics: enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC) [5]. Unfortunately, some strains, including *Escherichia coli* O157:H7 carry traits that can cause serious diseases in humans. *E. coli* O157:H7 is a member of the group of pathogenic *E. coli* strains enterohaemorragic (EHEC), verotoxinproducing (VTEC), verocytotoxigenic or shiga-toxin producing organisms (STEC). *E. coli* O157:H7 are characterized by the production of Shiga-toxins 1 and/or Shiga toxin 2, an essential virulence factor in the production of at least one Shiga toxin [4].

Food, a major trade commodity, represents an important vehicle for food-borne pathogen infections. Raw food, of animal origin, has been linked with food-borne outbreaks, and it has been widely reported that livestock animals are an important source of food-borne pathogens and that poultry, swine and cattle among others, are a reservoir for pathogens such as *Salmonella, Campylobacter, and E. coli* O157:H7 as well as other STEC [5].

For this reason we used to conduct a study on raw beef to determine the prevalence of *E. coli* O157.We focused on the use of VIDAS®technique in order to detect *E. coli* O157 after one day enrichment and then confirmation of the results conventionally by culturing on MacConkey Sorbitol, followed by biochemical tests and finally real-time PCR confirmation. This is the first study in Lebanon, especially in Tripoli, on the detection of the prevalence of *E. coli* O157 by different method in raw meat.

Material and Methods

Sample Collection

Eighty (80) raw meat samples from beef (minced meat) were collected from different slaughterhouses covered all Tripoli regions .Samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately placed into cooler boxes at 4±1˚ C and transported back to the laboratory. The samples were analyzed within two hours of collection. This study was realized in Health and Environmental Microbiology Laboratory – Lebanese University.

Detection of Pathogens Using Conventional Methods and VIDAS® Technique

Twenty-five gram of each sample (aliquots were taken from five locations in the package (four corners and the middle) to adequately represent the package) were placed in a sterile stomacher bag with 225 ml of Buffered Peptone Water (BPW), stomached for 2 minutes, and incubated at 7-24 hours at 41.5 ± 1°C. After incubation, the contents of the stomacher were mixed manually.

E. coli O157:H7 Preliminary Detection

An aliquot (0.5 ml) of BPW broth was placed into the sample well of the VIDAS®ECPT strip and heated for 5 ± 1 minutes using Heat and Go Operator. The strip was removed and left to cool for at least 10 minutes then placed in the VIDAS®instrument (Biomerieux-France) for 50 min with a VIDAS®ECPT solid phase receptacle (SPR). Results were analyzed according to the manufacturer's instructions. Positive results were confirmed immediately by culturing onto Sorbitol MacConkey agar (SMAC). Sorbitol negative colonies were identified using several biochemical testssuch as urea, indole and culturing on Kligler Ion Agar (KIA) (Bio-RAD, France). To confirm the biochemical results, a RapID™ ONE System (Remel, USA) was used.

PCR Confirmation of *E. coli O157:H7* Species

The Real-Tim PCR reactionwas used to detect the presence of genes encoding Shiga toxins (*stx1* &*stx2*) and *eae* gene coding the intimin (specific adhesin for *E. coli* O157:H7) using Real-Time PCR in CFX96 thermocycler (Bio-Rad-Germany). The DNA extraction was performed using QIAamp DNA MiniKit (Qiagen-Germany). The foodproof® STEC Screening LyoKit (Biotecon-Germany) was used. The instructions, protocols and PCR program were followed as given by manufacturer instructions.

Results

E. coli O157:H7 Detection

VIDAS®ECPT analysis revealed that 15 out of 80 (18.75%) raw beef meat were positive for *E. coli* O157:H7. After biochemical screening for the 15 VIDAS® positive strains, 12 were positive to glucose, lactose, and indole whereas the urea was negative. The RapID™ ONE System (Remel-USA) results showed that only 5 strains of the 15 VIDAS® positive strains were identified as *E. coli*. The Real-time PCR results showed that all of the 15 VIDAS strains were not exhibit the *stx1, stx2* and *eae* genes (Table 1).

Detection Methods	No. of positive samples/No. of sample tested	Percentage
VIDAS®	15/80	18.75%
Culture and biochemical test	5/15	33.33%
Real-Time PCR	0/5	0%

Table1: Results of E. coli detection by different methods.

Discussion

Human infections of *E. coli* O157:H7 have mostly been recognized to be from food products with animal origin [6]. The current study demonstrated (0%) prevalence of contamination with *E. coli* O157:H7 organisms in raw minced beef according to the real-time PCR method. The absence of VTEC O157 contamination appears compatible with the low prevalence of this pathogen in stool samples and low human *E. coli* O157:H7 infection cases in Tripoli.

The results of real-time PCR-based method were negative. However, other methods like VIDAS® system and conventional method gave positive results for *E. coli* O157:H7. The internal and positive control used in real-time PCR kit is often desired to rule out the possibility of false negatives. For this reason we gave our prevalence result (0%) depending on the real-time PCR analysis.

The discrepancy between results issued from different methods could be explained. The *E. coli* O157:H7 is not present in the minced beef samples, but both conventional methods and VIDAS® technique yielded false positive results. Therefore, the specificity of VIDAS was affected by the presence of contaminating bacteria in the enrichment broth.For conventional method, the selective medium of *E. coli* O157:H7 was based on the sorbitol-fermenting activity. But since many bacteria other than *E. coli* O157:H7, like *Shigella* spp., *Hafnia alvei* and *Edwardsiellatarda*, formed colorless colonies on sorbitol MacConkey agar, false positive results were shown by conventional method.

In comparison to other countries, the prevalence reported in this study is lower than that in previous studies. In Riyadh, Saudi Arabia, Ashgan et al., (2015) showed thatthe prevalence of *E. coli* O157H7in ground meat was (5%) [7]. Direct comparison of results is difficult due to differences in the study methodologies, such as sampling over a full year and the sampling of all regions in the country. In this study sampling occurred over 2 months only while in other studies it had occurred during 12 months-3 years, covering all the seasons.

In the study done by SimaHajian, et al. the highest prevalence of *E. coli* O157:H7 was found on meat sampled in fall and spring, which is in agreement with finding of previous studies on beef that reported peak prevalence occurs in early fall [8,9]. Time of sampling has an effect on the results, thus we should take this into consideration.

Conclusion

The overall objective of this study was to determine the prevalence of *E. coli* O157:H7 in raw minced beef in Tripoli. The results showed that *E. coli* O157:H7 was absent in the raw minced beef samples.

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References

1. WHO (2017) Food safety and foodborne illness.
2. CDC (2011) Estimates of Foodborne Illness in the United States.
3. Franco B, Landgraf M, Destro M, Gelli D (2003) Foodborne Disease in Southern South America. International Handbook of Foodborne Pathogens Marcel Dekker 733-743.
4. Meng J, Doyle M, Zhao T, Zhao S (2007) Enterohemorraghic *Escherichia coli*. Food Microbiology: Fundamentals and Frontiers 3rd (Edn.), 249-269.
5. Jay JM, Loessner MJ, Golden DA (2005) Foodborne Gastroenteritis. In, Modern Food Microbiology Springer pp: 619-655.
6. Jo MY, Kim JH, Lim JH, Kang MY, Koh HB, et al. (2004) Prevalence of characteristics of Escherichia coli O157 from major food animals in Korea. International Journal of Food Microbiology 95(1): 41-49.
7. Fantelli K, Stephan R (2001) Prevalence and characteristics of shigatoxin producing Escherichia coli and Listeria monocytogenes strains isolated from minced meat in Switzerland. International Journal of Food Microbiology 70(1-2): 63-69.
8. Hajian S, Rahimi E, Mommtaz H (2011) A 3-year study of Escherichia coli O157:H7 in cattle, camel, sheep,

goat, chicken and beef minced meat. International Conference on Food Engineering and Biotechnology 9: 162-165.

9. Bryane CM, Erol I, Call EJ, Kaspar CW, Burge DR, et al. (2003) Characterization of E. coli O157:H7 from downer and healthy dairy cattle in the upper Midwest region of the United States. Applied and Environmental Microbiology 69(8): 4683-4688.