Available online www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2016, 5(2):349-354

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Occurrence of coliforms, *E. coli* type 1 and pathogenic serovar O157:H7 in Iranian burgers

Siavash Maktabi[1], Zohreh Ahangari[2,3*] and Horyeh Mohammad Pour[4]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Box: 61355-145, Ahvaz, Iran
[2]Food and Drug Safety Evaluation Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran
[4]Graduated from faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran
**Email: ahangari.zohreh@yahoo.com*

ABSTRACT

Escherichia (E.) coli is an important microbiological sanitary indicator and O157:H7 is one of its serious pathogenic serovars in human. Based on an amendment issued by Iranian National Standards Organization at 2010, the acceptable level for presence of E. coli in raw frozen burgers was removed and producers allowed to made burgers with no obligation in this regard. This study investigated the prevalence and numbers of total coliforms, E. coli Type 1 and serovar O157:H7 in Iranian beef burgers which will be useful in a national monitoring program. Over a 6-month period, 200 frozen burger samples were purchased from 12 Iranian brands. All samples were subjected to MPN and direct plating on VRBA and subsequent biochemical tests for enumeration of coliforms and identification of typical E. coli. In addition, the samples were enriched in TSB with novobiocin and plating on CT-SMAC and finally confirmed by PCR to identify pathogenic serovar O157:H7. In addition, direct PCR assay was performed on all samples to identify possible target genes. E. coli Type1 was recovered from 32 samples with counts over $5x10^3$ cfu/g and total coliforms from 56 samples with more than $5x10^3$ cfu/g. E. coli O157:H7 was recovered from 1 sample (0.5%). Our data showed that some products may be unacceptable for consumption. It is recommended that based on national circumstances, the minimum allowable level of E. coli in burgers should be specified by the government authority to ensure public health. Also, a zero tolerance policies for E. coli O157 in meat products could be useful.

Key words: Coliforms, *E.coli* O157:H7, PCR, Burger, Iran

INTRODUCTION

Coliform bacteria are a group of Enterobacteriaceae family that lives in the intestinal tract of humans and animals. They are important microbiological sanitary indicators, which emphasizes hygiene in processing and handling of products. *Escherichia coli* is one of the most important species coliforms and potentially able to cause illness, either diarrhea or illness outside of the intestinal tract (Jay, 1992).

Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) is one of the most important of foodborne pathogens producing Verotoxin and has been traditionally associated with foodborne infection from consumption of foods with animal origin, particularly those originating from cattle, such as ground beef and burgers (Williams et al., 2000, Willshaw et al., 1994).

There have been several reports of isolation of the organism from meat products in different countries (Cagney et al., 2004, Chinen et al., 2009, Vernozy Rozand et al., 2002). Little is known about the prevalence of *E. coli* O157:H7 in ground beef and beef products in Iran (Sheikh et al., 2013).

Minced beef mostly used for production of burgers in Iran. Since this kind of products are marked as preservatives free, based on European standards the maximum tolerable level of *E. coli* in fresh ground beef and beef burgers is 500 and 5000 cfu/g respectively. According to the recommendations of the commission if the higher level than the limits was observed, it is necessary to temporarily halt production lines to improve the sanitary in factory and use good manufacturing conditions meats (European Commission, 2005).

Iranian National Standards Organization does not specifically state acceptable Enterobacteriaceae and coliforms numbers in minced meat and derivatives. According to the ISIR. No: 2304/ Act of 2009 the presence of *E. coli* in raw frozen burgers was unacceptable. However, based on ISIR NO: 2304 amendment No 1/2010 the limit totally was removed and producers allowed to made burgers with no obligation in this regard.

The aim of this study was to determine the numbers of coliforms, total *E. coli* type1 and serovar *E. coli* O157:H7 in Iranian burgers sold in Ahvaz supermarkets to compare the data with little previously reported, which will be useful in a national monitoring program. In this study, we used culture-based conventional methods in combination with PCR assay using 2 pairs of specific primers targeting H7, O157 genes for confirmation of *E. coli* O157:H7 isolated from burger samples. Also direct PCR assay was done on all samples to identify possible target genes.

MATERIALS AND METHODS

Sample preparation

In this cross-sectional survey, during a six month period a total of 200 frozen beef burger samples with different production date and batch number (30-35 sample/month), made by 12 producing company located in different provinces in Iran, were purchased from local distributors in Ahvaz city. Selected manufacturers were the major producer in their regions. Burger boxes were checked to be completely frozen and in shelf-life periods and then transferred to the laboratory under cool conditions and were subjected to microbiological analysis procedure as describe below.

Enumeration of coliforms and *E. coli* by colony-count technique

Samples were analyzed using the method described by ISO 4832:2006 (ISO 4832, 2006). Briefly, 10 g samples were homogenized by a stomacher (250 rpm/1 min) with 90 ml volumes of saline suspension and serial dilutions was made. Then 0.1ml aliquot of each dilution was spread on sterile petri dishes containing violet red bile glucose agar (VRBGA) (Merck). Plates were incubated at 37°C for 24 h and counted the pink and red colonies which presumptive coliforms. To enumerate *E. coli*, the plates containing at least 3-5 typical colonies were selected. Colonies were identify by subculturing into two tubes of brilliant green bile broth (BGBB) (Merck) containing a Durham tube, and into1% trypton water. Incubate on tube of BGBB at 37°C and the second tube of BGBB and the trypton water at 44°C for 24h. Colonies with gas production in both BGBB tubes with Indole positive were subcultured on Eosin methylene blue agar (EMB) (Scharlau, Spain) to check Green metallic shine and measured as typical *E. coli*.

Enumeration of coliforms and *E. coli* by most probable number technique (MPN)

MPN technique described by ISO 4831:2006 (ISO 4831, 2006) was used to enumeration coliforms and *E. coli* in parallel for all samples. A serial dilution of sample from 10^{-1} to 10^{-3} was prepared. 3 tubes in 3 sets of lauryl tryptose broth containing durham tube also were made. The tubes were inoculate with 1 ml of each diluted sample and incubate at 37°C for 24 h. Tubes showing gas production considered as positive coliform. The positive tubes were used to compute the number of coliforms using relevant table and also were subjected to calculate *E. coli* type1 by subculturing in BGBB and trypton water as described before.

Isolation and identification of *E. coli* O157:H7

All samples were examined for the presence of *E. coli* O157:H7 by regular procedure. 10g amount of each sample was added to 90 ml tryptone soy broth (TSB) (Quelab, Canada) supplemented with novobiocine (20 mg/L, Sigma) and homogenized. After incubated for 24 hours at 37˚C, 100µl suspension was spread onto Cefixime Telluride-Sorbitol MacConkey agar (CT-SMAC) (scharlau, Spain) supplemented with cefexime (0.25mg/ml) and tellurite potassium (2.5mg/ml). Plates were incubated for 24 hours at 37˚C and examined for typical *E. coli* O157:H7 colonies (colorless, circular with brown center colonies). The colorless colonies of non-sorbitol-fermenting presumptive *E. coli* O157:H7 were plated onto both Eosin-Methylene Blue agar (EMB) (Scharlau, Spain) and tryptone bile x-glucoronide (TBX) (Merck) and were incubated at 37 °C for 24 hours. White color colonies on TBX and displaying a green metallic shine on EMB as suspected strain to *E. coli* O157:H7 were subjected to PCR analysis.

The PCR procedures

DNA extraction

Extraction was performed using a modification of the method previously described by Lopez-Saucedo et al. (Lopez-Saucedo et al., 2003). Presumptive *E. coli O157:H7* colonies separately were grown overnight in 5ml TSB at 37 °C. These cultures were centrifuged (Hitachi 1110, Germany), the pellet was resuspended in 1 ml of sterile distilled water, and samples were heated at 100 °C for 10 min. After heating, the suspension was again centrifuged and the supernatant was used as the PCR template.

Polymerase chain reaction and electrophoresis

Presumptive colonies were subjected to PCR assay for amplification using 2 pairs of specific primers, including: *(FliC)* H7 and O157 according to the following program: initial denaturation at 94 ˚C for 3 min, and then 35 cycles comprising denaturation at 94 ˚C for 45 s, annealing at 60 ˚C for 45 s, and an extension at 72 ˚C for 60s. Following this, a final extension at 72˚C for 5 min was carried out. Each PCR tube contained 25 µl of reaction mixture, consisting of 2.5 µl PCR buffer (10x), 1.5 µl $MgCl_2$ (50 mM), 1 µl dNTP (10 mM), 0.5 µl Taq polymerase (2.5 U), 4 µl of a mixture of the 2 forward and revised primers (15 µM), 10.5 µl ddH2O and 5 µl of template extracted DNA. The mixture was then processed in a thermocycler (Bioer Technology Co., China). The targets, primer sequences and amplicon sizes for the PCR products are shown in Table 1.

The amplified PCR products were detected by electrophoresis (Paya pajoohesh, Iran) and staining and visualized under UV light illumination (UVT-20 SL, Iran).

Direct DNA extraction

To detect presumptive *E. coli* O157:H7 in burgers which may not be detectable by culture plating procedure, in parallel direct DNA extraction was performed. Briefly, 10g amount of each sample was added to 90 mL TSB and homogenized. After incubation for 24 hours at 37˚C, one milliliter of the enriched broth was centrifuged at 14,000 rpm for 3 min. Bacterial pellet was resuspended in 1mL of sterile normal saline and was subjected to boiling procedure for DNA extraction as described before.

RESULTS

The present survey focused on monitoring the enumeration of coli forms, *E. coli* type1 and pathogenic serovar O157:H7 in frozen Iranian burgers. Of the 200 burger sample tested, 56 were contaminated with coliforms more than $5x10^3$ cfu/g and 32 samples had *E. coli* more than $5x10^3$ cfu/g, which is higher than European standard. The levels of contamination of burgers to coliforms and *E. coli* are presented in Table 2.

E. coli O157:H7 was first isolated from 1 (0.5%) of total 200 burger samples by cultural procedure. Isolated strain then tested by PCR for the presence of O157 and (FliC) H7, the specific gene for genetic identification of *E. coli* O157: H7, and the results indicated that both gene were present in the strain (Fig. 1). However, no more strain was detected by direct DNA extraction method.

DISCUSSION

Burgers are popular RTE foods due to their convenience and richness in flavors. They can serve as vehicle for deleterious or pathogenic bacteria to people and may cause illnesses (Gormley et al., 2011). Unfortunately, burgers

have been rarely studied for their microbiological quality. Monitoring levels and presence of microorganisms is an important step in GMP and HACCP programs. For raw meat products such as burgers, safety and quality of final products can be estimated by the enumeration of indicator micro-organisms including *E. coli* and other coliforms (Jay, 1992). *E. coli* is a specific subgroup of coliforms which can indicate possible fecal contamination, inadequate heat treatment and poor hygienic condition of food handling (Akbarmehr and Khandaghi, 2012). Also in several reports stated that the burgers and minced beef are the most important vehicle for the transmission of pathogenic serovar O157:H7 (Williams et al., 2000, Willshaw et al., 1994).

This study was aimed to evaluate the level of indictor organisms such as coliforms, *E.coli* and the pathogenic serovar O157:H7 in burgers sold in Ahvaz (South-West Iran) supermarkets. Our data showed that more than 94.5% of samples are contaminated to the coliforms with different levels, whereas 56 samples (28%) have been contaminated to coliforms with more than $5x10^3$cfu/g. This finding clearly shows the poor sanitary condition in some factories. High contamination to typical *E. coli* also was observed where contamination of 32 samples (16%) exceeds the allowed maximum limit issued by European commission ($5x10^3$cfu/g).

The data could be compared with other studies around the world. For example in India, 22 (7.33%) samples (11 pork, 4 chicken, 4 beef and 3 carabeef) exceeded the limit for *E. Coli (*100/gram maximum)(Kumar et al., 2014). In another study in this country fecal coliform count from 1.8 - 5.3×10^6 in ready to eat meat products like beef tikka and beef sammi kabab. The author's emphasis that the most of the ready to eat meat products samples were considered to pose health risk to consumers and strict hygienic and sanitary measures are necessary during processing, storage and marketing (Kumar et al., 2011).

In a study the mean counts (CFU/g) of total mesophilic aerobic bacteria, the coliforms and *E. coli* in Turkish cig kofte (raw consumed spiced meatball) were 5.5×10^6, 2.9×10^3 and 2.6×10^2 respectively. Again authors wrote that the relatively high bacterial counts and status of harboring some foodborne pathogenic bacteria, particularly *E. coli* raise concerns regarding the hygienic quality of raw material and the handling process of RTE foods and its potential risks for public health (Cetinkaya et al., 2012).

In Iran, Tavakoli and Riazipour (Tavakoli and Riazipour, 2008) showed served meat cooked in resturents could be contaminated with coliform and pathogenic bacteria including *E. coli* and *S. aureus*. They reported 50% coliforms contamination of 216 samples examined in their study. They also reported *S. aureus* and *E. coli* contaminations 14.2% and 12.6% of samples such as grilled ground meat, grilled chicken and beef burger respectively. In another study microbiological quality of Bonab kebebs sold in Tabriz was studied and 1.69-5.3 Log (cfu/gr) was reported (Nemati et al., 2008).

In a study on meat products in West Azarbaijan province, 5% of samples were unacceptable according to the Iranian national standards (Kheyri et al., 2014). In Sanandaj (West of Iran) and In Ahvaz (South-West of Iran) the prevalence of *E. coli* in frozen food like beef burger 40% and 52%, respectively, was reported (Enayat et al., 2012). All of these results clearly support our results.

Also in our study serovar O157:H7 was isolated in one sample which indicates the risk of consumption of this kind of RTE food. Several studies have shown that *E. coli* O157:H7 and other STEC are present in meat products, mostly beef products. For example Cagney *et al*. (Cagney et al., 2004) investigated the prevalence and numbers of Escherichia coli O157:H7 in minced beef and beef burgers in supermarkets and butcher shops in the Republic of Ireland. Overall, *E. coli* O157:H7 was recovered from 43 samples (2.80%). In France Vernozy-Rozand et al. reported 0.12% (4/3450) samples positive for *E. coli* O157:H7 in large-scale processed minced beef (Vernozy Rozand et al., 2002). In another study in Argentina contamination of cooked and uncooked beef and chicken burgers and from chicken carcasses to *Escherichia coli* (STEC) O157:H7 was studied. Of the 24 STEC O157:H7 strains isolated, 20 were recovered from 19 (6.8%) out of 279 samples of beef and chicken burgers, and 4 strains from 4 (10.3%) out of 39 chicken carcasses (Chinen et al., 2009). Other studies found very different results, ranging from 16.8% (50/296) *E. coli* O157:H7 samples in Washington State, USA (Samadpour et al., 2002) to a study by Tarr et al. which did not recover the pathogen from 1400 retail ground minced beef samples from six stores in Seattle, USA (Tarr et al., 1999).

Few studies indicated the isolation of the bacterium in meat products in Iran. For example Rahimi (Rahimi et al., 2012) reported a high prevalence of *E. coli* O157 in beef samples (8.2%), followed by water buffalo (5.3%), sheep

(4.8%), camel (2.0%) and goat (1.7%). Again, in another study in Iran, high incidence of *E. coli* O157:H7 in ruminant's meat samples was reported by Momtaz. Where, 238 (29.02%) samples were positive for presence of *E. coli*. All of the isolates had more than one virulence gene including Stx1, Stx2, eaeA and ehly. (Momtaz et al., 2013). In South-West of Iran *E. coli* O157:H7 was found in 6(3.92%) of 153 sheep carcasses (Shekarforoush et al., 2008).

Similar to our study, in South- Western of Iran, the bacterium was recovered in 1% of burger samples. Authors indicated that beef burger could be a reservoir for *E.coli* O157:H7 in Khuzestan Province (Sheikh et al., 2013).

CONCLUSION

All above data showing that the burger may contaminate to pathogenic *E. coli* in high level. Also some paper indicates that the consumption of burgers is associated with *E. coli* O157 infections (Strachan et al., 2006). Although in the current study only 0.5% of the *E.coli* isolates were O157:H7 strains, but it has been reported that non-O157 strains could be verocytotoxigenic (Karmali, 1989, March and Ratnam, 1986).

This evaluation of the microbiological quality of Iranian burgers shows that some products may be unsafe and unacceptable for consumption. It is strongly recommended that good precautionary measures must be taken to prevent or reduce its contamination by microorganisms. To achieve this goal, only meat of good microbiological quality should be used for processing. More stringent monitoring program implement for preventing microbial contamination in burger production line. According to national circumstances, the minimum allowable level of *E. coli* in burgers should be specified by the government authority to ensure public health. A zero tolerance policy could be useful to control *E. coli* O157 in meat products in the country.

Acknowledgments
This study financially was supported by a grant (No. 5761) from vice chancellor for research, Ahwaz Jundishapur University of Medical Sciences. We would like to mention the greatly appreciation of Research Council of Shahid Chamran University of Ahvaz, Iran, for the laboratory support.

REFERENCES

[1] Akbarmehr, J. and Khandaghi, J. (2012), "A survey on the prevalence of Salmonella and coliforms in unpasteurized Iranian cheese using conventional culture method". *African Journal of Microbiology Research,* Vol. 6**,** pp. 968-971.
[2] Cagney, C., Crowley, H., Duffy, G., Sheridan, J. J., O'brien, S., Carney, E. and Bishop, R. H. (2004), "Prevalence and numbers of Escherichia coli O157: H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland". *Food Microbiology,* Vol. 21**,** No. 2, pp. 203-212.
[3] Cetinkaya, F., Mus, T. E., Cibik, R., Levent, B. and Gulesen, R. (2012), "Assessment of microbiological quality of cig kofte (raw consumed spiced meatball): Prevalence and antimicrobial susceptibility of Salmonella". *Food Control,* Vol. 26**,** No. 1, pp. 15-18.
[4] Chinen, I., Epszteyn, S., Melamed, C. L., Aguerre, L., Martinez Espinosa, E., Motter, M. M., Baschkier, A., Manfredi, E., Miliwebsky, E. and Rivas, M. (2009), "Shiga toxin-producing Escherichia coli O157 in beef and chicken burgers, and chicken carcasses in Buenos Aires, Argentina". *Int J Food Microbiol,* Vol. 132**,** No. 2, pp. 167-71.
[5] Enayat, K., Mansour, A., Nasrin, B., Mohammad, T., Mohammad, H. and Hanar, N. (2012), "Antibiotic resistance pattern in bacterial isolates obtained from frozen food samples of animal origin in Sanandaj and Ahvaz". *J Bacteriol Res,* Vol. 4**,** No. 3, pp. 38-41.
[6] European Commission (2005), "Commission Regulation (EC) no. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs". *Official J. Eur. Union,* Vol. L338**,** pp. 1-26.
[7] Gormley, F. J., Little, C. L., Rawal, N., Gillespie, I. A., Lebaigue, S. and Adak, G. K. (2011), "A 17-year review of foodborne outbreaks: describing the continuing decline in England and Wales (1992-2008)". *Epidemiol Infect,* Vol. 139**,** No. 5, pp. 688-99.
[8] Iso 4831 (2006), Microbiology of food and animal feeding stuffs- Horizontal method for the detection and enumeration of coliforms – Most probable number technique.
[9] Iso 4832 (2006), Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony-count technique

[10] Jay, J. M. (1992), *Modern Food Microbiology,* New York, NY, Van Nostrand Reinhold.
[11] Karmali, M. A. (1989), "Infection by verocytotoxin-producing Escherichia coli". *Clin Microbiol Rev,* Vol. 2**,** No. 1, pp. 15-38.
[12] Kheyri, A., Fakhernia, M., Haghighat-Afshar, N., Hassanzadazar, H., Kazemi-Ghoshchi, B., Zeynali, F. and Bahmani, M. (2014), "Microbial Contamination of Meat Products Produced in the Factories of West Azerbaijan Province, North West of Iran". *Global Veterinaria,* Vol. 12**,** No. 6, pp. 796-802.
[13] Kumar, P., Rao, J. and Haribabu, Y. (2014), "Microbiological quality of meat collected from municipal slaughter houses and retail meat shops from Hyderabad Karnataka region, India". *APCBEE Procedia,* Vol. 8**,** pp. 364-369.
[14] Kumar, S., Rindhe, S. N., Karle, S. D., Kumari, B., Ranjan, R. and Kumar, P. (2011), "Microbial quality of ready to eat meat product sold in Parbhani City (MS)". *Journal of Animal Research,* Vol. 1**,** No. 1, pp. 47-50.
[15] Lopez-Saucedo, C., Cerna, J. F., Villegas-Sepulveda, N., Thompson, R., Velazquez, F. R., Torres, J., Tarr, P. I. and Estrada-Garcia, T. (2003), "Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic Escherichia coli". *Emerg Infect Dis,* Vol. 9**,** No. 1, pp. 127-31.
[16] March, S. B. and Ratnam, S. (1986), "Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis". *J Clin Microbiol,* Vol. 23**,** No. 5, pp. 869-72.
[17] Momtaz, H., Safarpoor Dehkordi, F., Rahimi, E., Ezadi, H. and Arab, R. (2013), "Incidence of Shiga toxin-producing Escherichia coli serogroups in ruminant's meat". *Meat Sci,* Vol. 95**,** No. 2, pp. 381-8.
[18] Nemati, M., Ghorbanpour, H., Razavieh, S. V. and Hoseini, M. (2008), "Chemical composition and microbiological quality of the bonab kebabs sold in Tabriz market". *Journal of Food Safety,* Vol. 28**,** No. 3, pp. 315-323.
[19] Rahimi, E., Kazemeini, H. R. and Salajegheh, M. (2012), "Escherichia coli O157:H7/NM prevalence in raw beef, camel, sheep, goat, and water buffalo meat in Fars and Khuzestan provinces, Iran". *Vet Res Forum,* Vol. 3**,** No. 1, pp. 15-7.
[20] Samadpour, M., Kubler, M., Buck, F. C., Depavia, G. A., Mazengia, E., Stewart, J., Yang, P. and Alfi, D. (2002), "Prevalence of Shiga toxin-producing Escherichia coli in ground beef and cattle feces from King County, Washington". *J Food Prot,* Vol. 65**,** No. 8, pp. 1322-5.
[21] Sheikh, A. F., Rostami, S., Amin, M., Abbaspour, A., Goudarzi, H. and Hashemzadeh, M. (2013), "Isolation and identification of Escherichia coli O157: H7 from ground beef hamburgers in Khuzestan Province, Iran". *African Journal of Microbiology Research,* Vol. 7**,** No. 5, pp. 413-417.
[22] Shekarforoush, S., Tahamtan, Y. and Pourbakhsh, A. (2008), "Detection and frequency of Stx2 gene in Escherichia coli O157 and O157:H7 strains isolated from sheep carcasses in Shiraz-Iran". *Pak J Biol Sci,* Vol. 11**,** No. 8, pp. 1085-92.
[23] Strachan, N. J., Dunn, G. M., Locking, M. E., Reid, T. M. and Ogden, I. D. (2006), "Escherichia coli O157: Burger bug or environmental pathogen?". *International journal of food microbiology,* Vol. 112**,** No. 2, pp. 129-137.
[24] Tarr, P. I., Tran, N. T. and Wilson, R. A. (1999), "Escherichia coli O157:H7 in retail ground beef in Seattle: results of a one-year prospective study". *J Food Prot,* Vol. 62**,** No. 2, pp. 133-9.
[25] Tavakoli, H. R. and Riazipour, M. (2008), "Microbial quality of cooked meat foods in Tehran University's Restaurants". *Paki Med J,* Vol. 24**,** No. 4, pp. 595-599.
[26] Vernozy Rozand, C., Raygueniot, S., Ragot, C., Bavai, C., Mazuy, C., Montet, M. P. and Richard, Y. (2002), "Prevalence of Escherichia coli O157: H7 in industrial minced beef". *Letters in applied microbiology,* Vol. 35**,** No. 1, pp. 7-11.
[27] Williams, R. C., Isaacs, S., Decou, M. L., Richardson, E. A., Buffett, M. C., Slinger, R. W., Brodsky, M. H., Ciebin, B. W., Ellis, A. and Hockin, J. (2000), "Illness outbreak associated with Escherichia coli O157:H7 in Genoa salami. E. coli O157:H7 Working Group". *CMAJ,* Vol. 162**,** No. 10, pp. 1409-13.
[28] Willshaw, G. A., Thirlwell, J., Jones, A. P., Parry, S., Salmon, R. L. and Hickey, M. (1994), "Vero cytotoxin-producing Escherichia coli O157 in beefburgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain". *Lett Appl Microbiol,* Vol. 19**,** No. 5, pp. 304-7.