The Pharma Innovation Journal 2021; SP-10(12): 807-811

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 807-811

www.thepharmajournal.com
Received: 28-10-2021
Accepted: 30-11-2021

Mahantesh Meti
M.V.Sc. Scholar, Department of Livestock Products Technology, Madras Veterinary College, TANUVAS, Chennai, India

V Appa Rao
Dean, College of Food and Dairy Technology, Tamil Nadu, India

Bilifang Daimary
M.V.Sc. Scholar, Department of Livestock Products Technology, Madras Veterinary College, TANUVAS, Chennai, India

G Sundaresan
M.V.Sc. Scholar, Department of Livestock Products Technology, Madras Veterinary College, TANUVAS, Chennai, India

Param Debbarma
Ph.D. Scholar, Department of Livestock Products Technology, College of Veterinary Science, AAU, Khanapara, India

Corresponding Author
Mahantesh Meti
M.V.Sc. Scholar, Department of Livestock Products Technology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Prevalence of major food borne pathogens in ready-to-eat chicken products sold by street food vendors and fast-food outlets in Chennai city

Mahantesh Meti, V Appa Rao, Bilifang Daimary, G Sundaresan and Param Debbarma

Abstract
In present day food safety has become a major concern of human health which possess a great risk due to various food borne illness like diarrhoea, fever, shock etc. The present study reports the presence of food borne pathogens viz. *Staphylococcus aureus* and *Escherichia coli* from RTE chicken products collected from street food vendors and fast-food outlets in Chennai, India. The study showed that overall prevalence rate of *S. aureus* was 20% and 40% and for *E. coli*, it was 2.5% and 7.5% in RTE chicken products collected from street food vendors and fast-food outlets respectively. Notably, the prevalence rate was higher in RTE chicken products collected from fast-food outlets than street food vendors indicating more post handling- and cross contamination of the finished food product. Hence, mandatory periodical training concerning to food safety and quality before issuance of license to food vendors and time-to-time inspection by Food Safety/Designated Officer are to be considered.

Keywords: ready-to-eat, chicken, *S. aureus*, *E. coli*, fast food, street food, prevalence

1. Introduction
Most of the developing countries are experiencing increased urbanization resulting in growth of street food vendors and retail food outlets. The trend towards consumption of processed foods has changed with more people moving towards processed ready-to-eat (RTE) food products with chicken being the most preferred meat when compared to meat of other animals (Talukder 2020) [20]. The street foods are gaining popularity because of their accessibility, ready to eat, variety and nutritional value but sometimes they are perceived as unsafe because of poor handling practices during preparation of the finished product. Although these RTE chicken products are gaining popularity and demands, there is very few evidences about the microbiological quality and safety of these products which is of human health concern. The street food vendors are often very poor, uneducated and show little concern towards safe handling of foods (WHO, 2010) [22]. Most of the food-borne diseases reported in India are bacterial in origin and caused by bacteria such as *S. aureus*, *E. coli*, *Salmonella* and *Vibrio parahaemolyticus*. (NCDC 2017) [16].
Among the major food borne pathogens, *S. aureus* is isolated from wide range of food stuffs such as meat, cheese, milk and also from various environmental sources such as soil, dust, air or natural water (Kools and Schleifer, 1986) [12]. The food poisoning due to Staphylococcal enterotoxins occurs due to pyrogenic toxin superantigens which can retain their activity even after heat treatment (Fetsch and Johler, 2018) [4]. The outbreaks mainly occur due to improper handling of foods by personnel who are frequently contaminated with these organisms (Hatakka *et al*. 2000) [5]. *E. coli* is another important food borne pathogen causing diarrhoea, haemorrhagic colitis and life threatening haemolytic uraemic syndrome in humans (Wani *et al*. 2007) [21]. The contamination of chicken meat by *E. coli* is mainly associated with rupture of intestine during slaughtering process or due to cross-contamination during handling (Alonso *et al.* 2012) [1] and consumption of undercooked meat (Soon *et al.* 2011) [19]. With this background, the present study was undertaken to detect the prevalence of *S. aureus* and *E. coli* from RTE chicken products viz. grilled chicken, chicken 65, chilly chicken and tandoori chicken and to develop a suitable package of interventions to control these hazards.

2. Materials and Methods
2.1 Collection of samples
A total of 80 nos. of RTE chicken products consisting of four different chicken products viz.

chicken 65, chilli chicken, tandoori chicken and grilled chicken, were randomly collected in a sterile container from Chennai city for isolation and identification of *E. coli* and *S. aureus*. Among the 80 nos. of RTE chicken products, 40 nos. were collected from street food vendors and another 40 nos. were collected from fast-food outlets. Out of 40 nos. RTE chicken products collected from both street food vendors and fast-food outlets; 10 samples correspond to each of the four varieties of chicken products. The collected RTE chicken products were brought to the Meat Science Laboratory, Department of Livestock Products Technology, Madras Veterinary College and were analysed.

2.2 Reference bacterial strains

E. coli (MTCC 41) and *S. aureus* (MTCC 3160) were procured from CSIR-IMTECH, Chandigarh and were used in this study.

2.3 Bacteriological Media

Buffered peptone water, Brain Heart Infusion (BHI) broth, Tryptone soya broth (TSB), MacConkey agar, Eosin Methylene Blue (EMB) agar, Mannitol salt agar (MSA), Baird parker agar (BPA), Peptone water, Agar agar, Nutrient broth were procured in dehydrated form from M/S Hi-Media, Mumbai. All the media were used as per the manufacturer's instructions.

2.4 Vitek®2 compact instrument

Vitek®2 compact instrument, Vitek saline (0.45% sodium chloride), Gram positive colorimetric reagent cards (GP cards), Gram negative colorimetric reagent cards (GN cards), stock cultures, sterile swab or applicator stick (Himedia), clear plastic (polystyrene) test tube, DensiChek turbidity meter, Densicheck standards of (0.0, 0.5, 0.8 McFarland), bar coded cassettes to hold and incubate the cards were used for analysis.

2.5 Materials for Polymerase Chain Reaction (PCR)

Qiagen® bacterial Deoxyribonucleic acid (DNA) extraction mini kit for extraction of DNA was obtained from M/S Synergy Scientific Services. Both forward and reverse primers targeting genes specific to organisms investigated in this study were custom designed and obtained from M/S Synergy Scientific Services.

2.6 Microbiological analysis

2.6.1 Isolation of *S. aureus*

Detection and identification of *S. aureus* was done as per the ISO standard 6888-1:1999 [10] and 6888-2: 1999 [11]. Briefly, pre-enrichment of 25 gm sample was done by adding 225 ml of sterile buffered peptone water in stomacher bag and homogenized at 230 rpm for 30 seconds using stomacher. The homogenate of 1 ml was inoculated into BHI broth supplemented with 6% NaCl and incubated at 37 ˚C for 24 hours. After incubation, selective plating was done by taking a loop of inoculum from BHI broth and was streaked on surface of sterile MSA and BPA, followed by incubation at 37 ˚C for 24 hours. The suspected isolates were then picked for biochemical analysis to confirm the microorganism.

2.6.2 Isolation of *E. coli*

Detection of *E. coli* was done as per the ISO standard 16654:2001[9]. Briefly, pre-enrichment of 25 gm sample was done by adding 225 ml of sterile buffered peptone water in stomacher bag and homogenized at 230 rpm for 30 seconds using stomacher. The homogenate of 1 ml was inoculated into TSB and incubated at 37 ˚C for 18-24 hours. After incubation for 24 hours, selective plating was done by taking a loop of inoculum from TSB and streaked on surface of sterile MacConkey agar and EMB agar followed by incubation at 37 ˚C for 24-48 hours. The suspected isolates were then picked for biochemical analysis to confirm the microorganism.

2.7 Analysis by Vitek®2 system

The reference isolates (MTCC 41, MTCC 3160) and positive isolates from RTE chicken products were propagated by sub-culturing on Nutrient Agar and Soyabean Casein Digest Agar at 37 ˚C for 18-24 hours. Bacterial suspension was prepared with density of 0.50 to 0.63 McFarland (McF) standard using Vitek saline and density was adjusted using Vitek Densi Check instrument. Based on the gram staining, gram positive and gram-negative identification cards were selected for gram positive and gram-negative organism respectively. The cards and bacterial suspension kept in plastic racks were manually inserted in the VITEK 2 system reader incubator module (incubation temperature 35.5 °C). The cards were automatically filled by vacuum device and were automatically sealed and subjected to kinetic fluorescence after every 15 minutes. The results were interpreted by comparison with data base after incubation and final results were obtained after 4-8 hours of incubation time. All the cards were discarded as per the standard operating procedure.

2.8 Analysis by PCR

Specific primers for the partial amplification of conserved genes of *S. aureus* (nuc) and *E. coli* (uspA) were used for molecular detection of food borne pathogens by PCR. Template DNA was extracted from overnight broth cultures of *S. aureus* and *E. coli* by boiling method. 2 (two) ml of overnight culture was centrifuged at 13,000 rpm for 3 minutes to obtain bacterial pellet which was resuspended and centrifuged thrice in phosphate buffer saline (pH 7.2). Then, it was further suspended in 50 µl of nuclease free water and boiled for 10 minutes and placed immediately on ice. The suspension was centrifuged for 2 minutes at 13,000 rpm and the supernatant is transferred in a new micro-centrifuge tube from which 2 µl was used template DNA for analysis by PCR.

The species-specific primers were used to amplify the DNA of *S. aureus* by targeting its *nuc* gene (Hedge, 2013) [6] and for *E. coli*, the gene specific primers were used to amplify the DNA by targeting its *uspA* gene (Chen and Griffiths, 1998) [3] (Table 1). The PCR reaction mixture and cycling conditions were optimized to amplify the DNA of *S. aureus* and *E.* coli. The PCR products were then analyzed using 1.5% agarose gel electrophoresis and recorded using gel documentation system.

2.9 Statistical analysis: The data were analyzed by suitable statistical methods.

3. Results and Discussion

3.1 Prevalence of S. aureus in RTE chicken products

From the present study we have observed that the overall prevalence of *S. aureus* in RTE chicken products from street food vendors was 20% and from fast-food outlets was 40%. Interestingly, the incidence of *S. aureus* prevalence was found in tandoori chicken and grilled chicken only among all the RTE chicken products in case of street food vendors.

However, all the RTE chicken products of fast-food outlets showed contamination with *S. aureus.* (Table 2 and 3). The results were in agreement with the findings of Mashak *et al*. (2015) [14] who reported 35% and 40% prevalence of *S. aureus* in cooked and semi cooked RTE chicken products in Tehran. In previous study of Manguiat and Fang (2013) [13], they reported the prevalence of *S. aureus* up to 17% in hot grilled chicken samples collected from street foods in Philippines. In contrary, Campos *et al*. (2015) [2] reported zero incidence of Staphylococcus in hot dogs and hamburgers in Portugal. In similar study, Meldrum *et al*. (2005) [15] also found less than 0.5% incidence in long term surveillance in United Kingdom. The results of positive isolates from RTE chicken products were validated by VITEK®2 System (Table 4), which were in agreement with findings of Odumeru *et al*. (1999) [17] who recorded 95% of sensitivity and 95% specificity for identification of *S. aureus*. The identification by Vitek®2 system to species level was 100% for *S. aureus*. The results were further confirmed by PCR in which bands of 181 base pairs(bp) were observed for *S. aureus* in 1.5% agarose gel (Fig. 1). Based on our findings, it may be concluded that cutting or slicing and garnishing of the finished product by the food handlers while serving might have led to cross-contamination with *S. aureus.*

3.2 Prevalence of *E. coli* in RTE chicken products

The other important aspect of our study was to find out the prevalence of another major food borne pathogen i.e., *E. coli* in RTE chicken products from street food vendors and fast-food outlet. Here we observed that the overall prevalence of *E. coli* in RTE chicken products from street food vendors was 2.5% whereas from fast-food outlet was 7.5% (Table 2 and 3). The result was higher than the findings of Meldrum *et al*. (2005) [15] who had reported an incidence of 1.6% for *E. coli* in United Kingdom from various RTE food products. However, Manguiat and Fang (2013) [13] found prevalence of 13% of *E. coli* in hot grilled chicken samples collected from street vended foods in Philippines.

The prevalence of *E. coli* in RTE chicken products from fast-food outlet was higher than that of street food vendors. Similar types of findings were also reported by Hussain and Sarwar (2014) [8] in a study carried out at Faisalabad where they reported an incidence of (6.24%) for *E. coli* in RTE fast foods. Hosein *et al*. (2008) [7] reported 4.5% of incidence of *E. coli* in RTE products and the presence was mainly attributed to water, which acted as source for cross contamination of these organisms. Sharaf and Sabra (2012) [18] found 25% of prevalence of *E. coli* in chicken luncheon and 20% in chicken shawerma collected from different supermarket tin Al-Taif.

The results of positive isolates from RTE chicken products were validated by VITEK®2 System (Table 4), which were in agreement with findings of Odumeru *et al*. (1999) [17] who recorded 100% of sensitivity for identification of *E. coli*. The positive RTE chicken product which were confirmed by

Table 1: Primer sequence for PCR

Sl. No	Target gene	Primer sequence	Product size (bp)	References
1.	*uspA*	*E. coli* Forward Primer 5' CCG ATA CGC TGC CAA TCA GT3' *E. coli* Reverse Primer 5' ACG CAG ACC GTA GGC CAG AT 3'	884	Chen and Griffiths (1998) [3]
2.	*nuc*	*S. aureus* Forward Primer 5' GTGCTGGCATATGTATCGCAATTGT3' *S. aureus* Reverse Primer 5' TACGCCCTTATCTGTTTGTGATGC3'	181	Hedge (2013) [6]

Table 2: Prevalence of *S. aureus* and *E. coli* in RTE chicken products from street food vendors in Chennai city

Sl. No	Product	Number of samples screened	Number of positive samples	
			S. aureus	***E. coli***
1.	Chicken 65	10	0	0
2.	Chilly chicken	10	0	1(10%)
3.	Tandoori chicken	10	4(40%)	0
4.	Grilled chicken	10	4(40%)	0
Total		40	8(20%)	1(2.5%)

Table 3: Prevalence of *S. aureus* and *E. coli* in RTE chicken products from fast food outlets in Chennai city

Sl. No	Product	Number of samples screened	Number of positive samples	
			S. aureus	***E. coli***
1.	Chicken 65	10	1(10%)	2(20%)
2.	Chilly chicken	10	4(40%)	0
3.	Tandoori chicken	10	6(60%)	1(10%)
4.	Grilled chicken	10	5(50%)	0
Total		40	16(40%)	3(7.5%)

Table 4: Identification of food borne pathogens using Vitek®2 System

Sl. No	Reference positive isolates tested (Genus)	Vitek®2 system identification	Identification level	Sensitivity (%)
1.	*S. aureus*	*S. aureus*	Excellent	100
2.	*E. coli*	*E. coli*	Excellent	100

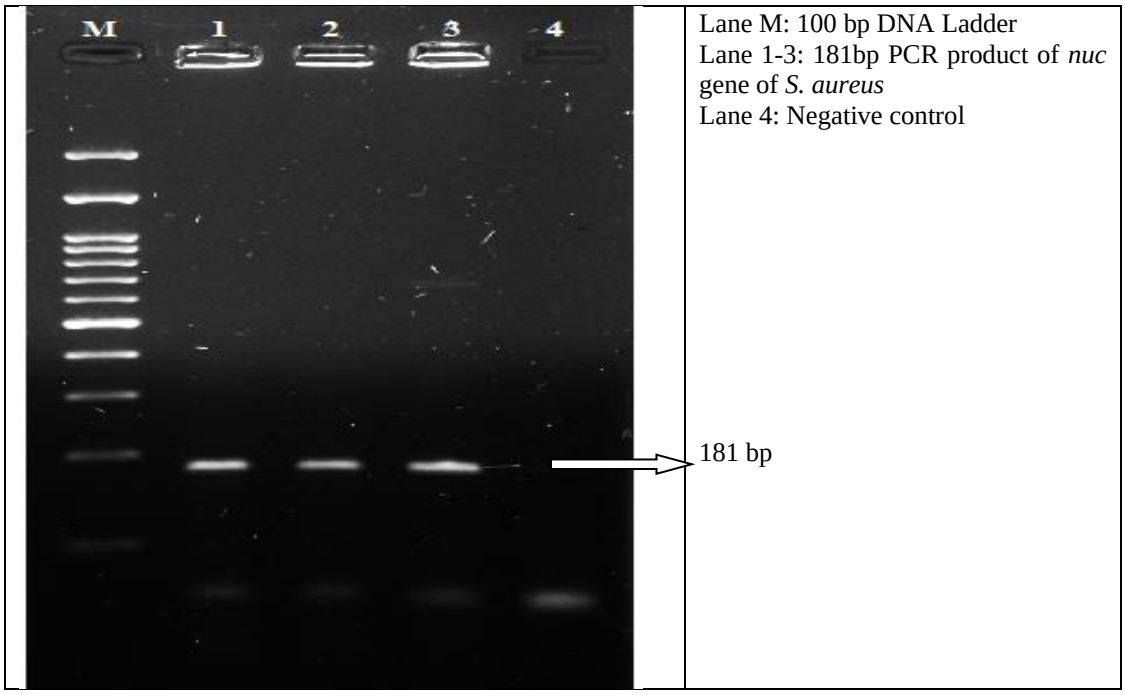

Fig 1; PCR product of *S. aureus*

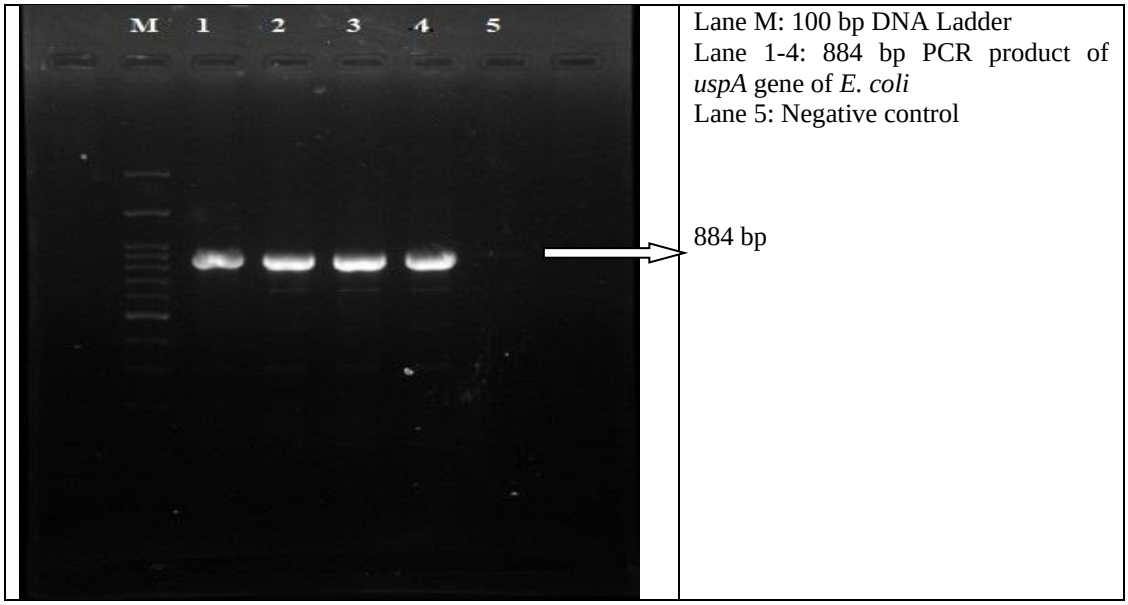

Fig 2: PCR product of *E. coli*

biochemical tests and VITEK®2 system was further analysed by PCR. The confirmation of *E. coli* targeting *usp*A gene was validated with amplified product size of 884 base pair by PCR and visualized in 1.5% agarose gel electrophoresis (Fig 2). Hence, from the above findings it may be concluded that the presence of *E. coli* in finished RTE chicken products might be due to the use of contaminated water for cooking and washing of utensils and accessories like knives, chopping board etc.

4. Conclusion

This study regarding microbiological quality of RTE chicken products sold by street food vendors and fast-food outlets in Chennai city detected the presence of *S. aureus* and *E. coli* which may pose human health hazard. Moreover, it was noted that higher the post-handling of finished RTE chicken products, greater is the chance of contamination with food borne pathogens. Considering the facts, it is recommended to create awareness among the street food vendor and fast-food outlets regarding the hygienic practices for preparation of RTE finished food products. Also, periodical monitoring by concerned authority might curb down the contamination of RTE food products with food-borne pathogens for the wellbeing and safety of the consumers.

5. References

1. Alonso MZ, Lucchesi PMA, Rodríguez EM, Parma AE, Padola NL. Enteropathogenic (EPEC) and Shigatoxigenic *Escherichia coli* (STEC) in broiler chickens and derived products at different retail stores. Food Control 2012;23(2):351-355.
2. Campos J, Gil J, Mourao J, Peixe L, Antunes P. Ready-to-eat street-vended food as a potential vehicle of bacterial pathogens and antimicrobial resistance: an exploratory study in Porto region, Portugal. International journal of food microbiology 2015;206:1-6.
3. Chen J, Griffiths MW. PCR differentiation of

Escherichia coli from other Gram negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. Letters in applied microbiology 1998;**27**(6):369-371.
4. Fetsch A, Johler S. Staphylococcus aureus as a foodborne pathogen. Current Clinical Microbiology Reports 2018;5(2):88-96.
5. Hatakka M, Björkroth KJ, Asplund K, Mäki-Petäys N, Korkeala HJ. Genotypes and enterotoxicity of Staphylococcus aureus isolated from the hands and nasal cavities of flight-catering employees. Journal of food protection 2000;63(11):1487-91.
6. Hedge. Identification and characterization of *Staphylococcus aureus* and *Streptococcus* in bovine mastitis milk. Thesis submitted to Karnataka Veterinary Fishery and Animal Sciences University, Bidar, Karnataka 2013.
7. Hosein A, Muñoz K, Sawh K, Adesiyun A. Microbial load and the prevalence of *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. in ready-to-eat products in Trinidad. The Open Food Science Journal 2008;(2):23-28.
8. Hussain I, Sarwar A. Bacteriological examination of some ready-to-eat foods in Faisalabad. J Glob. Innov. Agric. Soc. Sci 2014;2(2):93-96.
9. ISO. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Escherichia coli* O157. ISO Norm 16654:2001. International Standardization Organization ed., Geneva, Switzerland 2001.
10. ISO 6888-1:1999 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)-Part 1: Technique using Baird-Parker agar medium. International Standardization Organization ed., Geneva, Switzerland.
11. ISO 6888-2:1999. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)-Part 2: Technique using rabbit plasma fibrinogen agar medium. International Standardization Organization ed., Geneva, Switzerland.
12. Kools WE, Schleifer KH. Bergey's Manual of Systematic Bacteriology. Edn. 1st, Vol 2, The Williams and Wilkins Co. Baltimore, 1986, 1013-1035.
13. Manguiat LS, Fang TJ. Microbiological quality of chicken-and pork-based street-vended foods from Taichung, Taiwan, and Laguna, Philippines. Food microbiology 2013;36(1):57-62.
14. Mashak Z, Langroodi AM, Ehsani A, Ilkhanipoor A, Fathabad AE. Microbiological quality of ready-to-eat foods of Tehran province. African Journal of Food Science 2015;9(5):257-261.
15. Meldrum RJ, Ribeiro CD, Smith RMM, Walker AM, Simmons M, Worthington D *et al*. Microbiological quality of ready-to-eat foods: results from a long-term surveillance program (1995 through 2003). Journal of food protection. 2005;68(8):1654-1658.
16. NCDC 2017. https://ncdc.gov.in/WriteReadData/linkimages/CD%20Alert4053017156.pdf.
17. Odumeru JA, Steele M, Fruhner L, Larkin C, Jiang J, Mann E *et al*. Evaluation of accuracy and repeatability of identification of food-borne pathogens by automated bacterial identification systems. Journal of clinical microbiology 1999;37(4):944-949.
18. Sharaf EM, Sabra SM. Microbiological loads for some types of cooked chicken meat products at Al-Taif Governorate, KSA. World Applied Sciences Journal. 2012;17(5):593-597.
19. Soon JM, Chadd SA, Baines RN. Escherichia coli O157: H7 in beef cattle: on farm contamination and pre-slaughter control methods. Animal Health Research Reviews 2011;12(2):197-211.
20. Talukder S, Mendiratta SK, Kumar RR, Soni A, Bardhan D. Evaluation of meat consumption pattern and meat quality in North Indian cities. Journal of Animal Research 2020;10(3):365-373.
21. Wani SA, Hussain I, Nabi A, Fayaz I, Nishikawa Y. Variants of eae and stx genes of atypical enteropathogenic *Escherichia coli* and non-O157 Shiga toxin-producing *Escherichia coli* from calves. Letters in applied microbiology. 2007;45(6):610-615.
22. WHO 2010. https://www.who.int/foodsafety/fs_management/No_03_StreetFood_Jun10_en.pdf.