Iranian Journal of Military Medicine Winter 2012, Volume 13, Issue 4; 207-212

Bacteriological quality of ready to eat food in four military restaurants

Tavakoli H. R.[1] ***PhD***, **Farhang K.**[*] ***BSc***, **Karimi Zarchi A. A.**[2] ***PhD***, **Heydari E.**[3] ***BSc***

[*]*Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran;*
[1]*Health Research Center, Baqiyatallah Institute of Medical Sciences, Tehran, Iran;*
[2]*Department of Statistics & Epidemiology, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran;*
[3]*Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran*

Abstract

Aims: Random sampling and performing bacteriological tests are of the most important methods for examining the bacteriological quality of foods. The aim of this study was to evaluate the bacteriological quality of ready-to-use foods and salads in four restaurants affiliated to a military center in Tehran.

Methods: This cross-sectional study was carried out in 2009. 288 food and salad samples from four restaurants affiliated to a military center were chosen randomly and examined for Total Bacterial Count, Coliform bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* using standard methods. Results were analyzed by SPSS 15 software using descriptive statistical indices and ANOVA test.

Results: The highest Total Bacterial Count was detected in the samples of restaurant B and the highest Coliform bacteria, *Escherichia coli* and *Staphylococcus aureus* contamination was detected in the samples of restaurant A. 5 samples had *Escherichia coli* contamination, but no *salmonella* contamination was observed in tested samples.

Conclusion: Bacterial contamination in some served foods and salads of the studied restaurants is more than standard level and regular control of these restaurants along with complementary studies is essential.

Keywords: Bacteriological Survey, Food, Salad, Restaurant

Introduction

Today, the diseases result from food are considered as one of the major health problems in different countries and are occurring repeatedly in advanced and industrial countries so that in the U.S., nutritional diseases are of secondary importance after respiratory and lung diseases [1]. For example, according to *Hensey C.A* and *Ryne C.A*, consumption of the dairy products contaminated with salmonella leads to two epidemics in the United States during which 170 and 224 individuals were poisoned respectively and *Salmonella enteritidis* and *Salmonella typhimurium* were introduced as their agent [2, 3].

According to the report of Centers for Disease Control (CDC), about 77% of infections and food poisoning occur in restaurants, 20% in homes and 3% from commercial foods that non-compliance with health standards and the secondary pollution are the cause for the majority of them [4]. Among different bacteria agents of *Staphylococcus aureus*, salmonella, *Escherichia coli* and *Campylobacter jejuni* are among the most common bacterial causing infections and food poisoning. According to the report of the U.S. Food and Drug Administration (FAD), nearly, 46% of food infections in this country during 1997 to 2007 were due to Salmonella, and it is known as most important factor of food infections in England too [6, 5].

In Iran, although there are no statistics on the incidence of infections and food poisoning, but undoubtedly due to unfavorable conditions of the production, storage, distribution and consumption of food that is often without control of responsible organizations infection is more (in Iran) than developed countries. According to many researchers, one important way to ensure food safety, is implementation of HACCP systems in food preparation and distribution centers, including restaurants which are supplying foods that in order to ensure consumer health, the emphasis is on the implementation of these controlling systems [7,8].

According to the standard, microbial tests which are conducted on ready-to-use foods are varied according to the type of food but mostly include total bacterial count, Coliform, *Staphylococcus aureus*, mold and yeast and searching for some pathogenic bacteria like Salmonella, *Escherichia coli* and *Listeria monocytogenes* [9].

The importance of determination of total bacterial count, numbers of Coliform in cooked and ready-to-use foods, is in the indication the secondary contamination and lack of health standards especially personal hygiene and the importance of searching for pathogenic bacteria is in the prevention of infections and food poisoning. Therefore, the microbiological

* Correspondence; Email: farhangkazem@yahoo.com
Received 2010/10/29; Accepted 2012/01/06

control of ready-to-use foods is very important and absolutely necessary [10, 6, 4].

Kilic who has studied the status of meat food products in Turkey in the past decade believes that safety and quality of the foods supplying in restaurants is very important due to prevention of infections and food poisoning, and restaurant managers must establish confidence in their customers [11].

There are many reports regarding microbial contamination of salads and ready-to-use foods and their contamination with pathogenic bacteria in different parts of the world which we can point out to *Lin* studies in U.S [12], *Soriano* in Spain [13], *Ripabelli* in Italy [14], *Fang* in Taiwan, *Ghadge* in India [16], *Meldrum* in England [17], *Froder* in Brazil [18], *Bahk* in South Korea [19], *Abodias* [20], *Abougrain* in Libya [21] and *Gormley* in England [23].

Christison et al. in their microbiological study investigated 4 food supplying centers in Johannesburg, South Africa. They choose 163 samples of foods, salads, utensils, cutting boards and the hands of staffs in the kitchen randomly and conducted total bacterial count, Coliform, *Staphylococcus aureus*, and salmonella, *Listeria monocytogenes* and *Bacillus cereus* search on all of them. Contamination with Salmonella and Listeria was confirmed 16 and 4% of ready-to-use foods respectively [24].

According to Food Codex Commission of World Health Organization classification, cooked and ready-to-use foods and different kind of salads are among "High risk foods" classification. Foods like ground-meat kebab, chicken and different kind of salads are known as the most vulnerable nutrition for microbial growth and most common foods causing food poisoning that the available reports confirm this issue. Therefore, sanitary control of such foods in preparation and supply centers, especially in restaurants is very important [12, 14, 16, and 18].

Results of studies in Iran show that hygienic quality of prepared foods and salads is not desirable and microbial contamination of some of these foods is higher than standard [25, 26]. This issue is more important, especially in academic and military centers because their staffs eat at least one meal in their workplace. In a study by *Salek* in Shadid Beheshti University's affiliated medical centers conducted on 100 samples of meat foods and 34 samples of consumption salads regarding total bacterial count, Coliform count and contamination with *Staphylococcus aureus*, Salmonella and *Clostridium perfringense*, contamination to Coliform more than standard is confirmed in samples of salads, ground-meat kebab and chicken. In this study *Staphylococcus aureus* and *Clostridium perfringense* were isolated from 61 and 4 samples respectively [25]. In *Tavakoli* and *Riazipour* study sample foods from 6 Health centers affiliated to one of the Medical Universities in Tehran were evaluated from microbiological point of view that some ready-to-use foods (such as Ground-meat kebab) had microbial load higher than standard and contamination of 38.9 and 55.6% to *Staphylococcus aureus* and *Escherichia coli* was confirmed [26]. This issue is not restricted to Iran and as pointed out before, it is reported in many countries around the world. In a study by *Soriano* et al. on 342 samples from 2 kinds of food served in 19 university restaurants in Valensia, the average contamination to *Escherichia Coli* was determined as 1.7 and 8.8% respectively and the average contamination to *Staphylococcus aureus* as 3.5 and 7.6% respectively [13].

During the several years passing from the activity of restaurants contracting with one of the military centers in the homogenizing plan, bacterial quality of offered foods and salads has not been studied so far and considering that many of staffs of this military center and their families (considering 50% discount) are using at least once a month from this restaurant, this study was done in order to ensure the safety of ready-to-use foods and salads in these restaurants.

Therefore, the aim of this study was to evaluate the bacteriological quality of ready-to-use foods and salads at 4 restaurants affiliated to a military center in Tehran.

Methods

In this cross sectional study, in compliance with the standards no. 8923-1, 9263, 6806 and 1810 of the Iran standard national institution, the sampling was done out of three much-used and dangerous foods (ground-meat kebab, Bakhtiari Kebab and fish) and salads in four restaurant contracting with a military center in north, east, west and south parts of the Tehran whose name are presented as A, B, C, D, within the three time period with two-month interval.

After a review of the literature and similar studies and internal and external sources in order to use their methods and results, first necessary coordination was made with welfare service management which above mentioned restaurants were covered by them and the managers of 4 desired restaurants for sampling. In order to observe the ethical principles of the study, it was explained to restaurant managers that the study results will be given confidentially to them and when the results are published, the name of any restaurants

will not be mentioned.

At each visit of restaurants, of each of the three types of food and salads served, 6 samples were randomly sampled. Then collected samples were transferred to laboratory of Health Department in a special container at cool conditions, and general and specific bacteriological tests have been performed based on advised method by American Public Health Association (APHA) and Iranian national standards on all of the samples [10, 4].

To count Coliform, first off, samples were cultured in Briliant Green Bile Broth and VRBA media and after 24 to 48 h of pressing, counting was made. If the gas in brilliant green tubes is created and red-violet colonies are emerged in VRBA environment then the result of the test is positive and the sample is recognized as contaminated with Coliform [7, 4].

For identifying *Escherichia Coli*, Eekman test was used. In this test, the sample was cultured in green broth and peptone water and after 24 hours of incubation, drop of the Kovacs reagent was added to tubes containing peptone water medium. If a red color ring is made on the tube, the sample is considered positive from *Escherichia coli* point of view that this diagnosis was confirming by IMVIC test.

For identifying and counting *Staphylococcus aureus*, the surface culture method of Staphylococcus in specific environment of Baired Parker was used. For counting *Staphylococcus aureus*, black colonies, which a light halo was around them, were counted and Coagulase Test was used too.

For identifying salmonella after enrichment in selective and non-selective media such as lactose broth, Tetrathionat broth, Selenite sistin broth, the samples were cultured in Salmonella-Shigella agar selective solid environments (SSA) and bright green agar (BGA) in linear manner. Then doubtful colonies were transferred to differential culture environments TSI, Lysine Iron Agar and urea and were tested for the presence or absence of Salmonella [10, 7, 1]. Finally, the results of these experiments were compared with the standard tables of National Institute of Standards and Industrial Research and Ministry of Health regarding permissible contamination of foods.

The data was recorded in the computer and was described using SPSS 15 software and descriptive statistic (determination of percent and average). Considering the equality of variances and normal distribution of the measured variables by doing single-sample test (average compared with a standard sample) and the average of several samples, the results were analyzed by ANOVA statistical test.

Table 1- The comparison of total bacterial count, Coliform and *Staphylococcus aureus* and contamination with *Escherichia coli* in salad and food samples tested

Contamination→ Food type↓	Samples No.	The average of bacterial count (cfu/g)	The average of Coliform count (cfu/g)	The average of *Staphylococcus aureus* count (cfu/g)	Positive cases of *Escherichia coli*
Ground-meat kebab	72	$1.55\times10^{4}(\pm1.07\times10^{2})$	$9.42\times10^{2}(\pm1.68\times10^{1})$*	$6.40\times10^{1}(\pm7.30)$*	2 (2.77%)
Bakkhtiyari Kebab	72	$2.15\times10^{4}(\pm2.68\times10^{2})$	$8.02\times10^{2}(\pm1.36\times10^{1})$*	$7.80\times10^{1}(\pm1.33)$*	0
Fish	72	$7.62\times10^{3}(\pm1.55\times10^{3})$	$1.22\times10^{2}(\pm1.55\times10^{1})$*	$2.39\times10^{1}(\pm1.55)$*	0
Salad	72	$1.17\times10^{5}(\pm1.93\times10^{2})$	$4.04\times10^{3}(\pm7.88\times10^{1})$*	$1.47\times10^{2}(\pm1.47)$*	3 (4.16%)
Total	288	-	-	-	5 (1.73%)

*Contamination more than standard

Table 2- The comparison of the average of *Staphylococcus aureus* in salad and food samples tested in terms of food type and restaurant (cfu/g). Contamination in all cases was more than standard.

Food type→ Restaurant↓	Ground-meat kebab	Bakhtyari Kebab	Fish	Salad
A	$1.50\times10^{2}(\pm1.04\times10^{1})$	$4.10\times10^{1}(\pm3.30)$	$2.09\times10^{1}(\pm2.56)$	$3.60\times10^{2}(\pm2.19)$
B	$5.10\times10^{1}(\pm5.10)$	$1.83\times10^{2}(\pm2.65\times10^{1})$	$1.15\times10^{1}(\pm1.04)$	$2.20\times10^{2}(\pm1.58)$
C	$2.60\times10^{1}(\pm1.20)$	$5.20\times10^{1}(\pm3.30)$	$3.55\times10^{1}(\pm4.36)$	$2.27\times10^{2}(\pm1.05)$
D	$2.90\times10^{1}(\pm1.90)$	$3.50\times10^{1}(\pm1.70)$	$2.78\times10^{1}(\pm2.29)$	$1.63\times10^{2}(\pm6.60)$
Average	$6.40\times10^{1}(\pm7.30)$	$7.80\times10^{1}(\pm1.33)$	$2.39\times10^{1}(\pm2.58)$	$1.47\times10^{2}(\pm1.47)$

Results

Regarding total microbial count, Coliform count and *Staphylococcus aureus* count, the highest contamination was found in samples of salads and the lowest in fish samples (Table 1). Meanwhile, of 288 food samples tested, 5 samples (1.73%) contamination to *Escherichia coli* is confirmed, out of which 3 cases were related to salad samples (60% cases positive) and 2 cases (40% cases negative) to ground-meat kebab. In none of 288 samples tested, Salmonella infection was not confirmed. Also the average of Coliform count, *Staphylococcus aureus* count and *Escherichia coli* were higher than standard (Table 1). Statistically, the

difference between the total count of bacteria in the salad and fish samples was significant ($p<0.05$) but its difference was not significant with Ground-meat kebab and Bakhtiari kebab.

The average of *Staphylococcus aureus* count in salad and food samples tested in terms of food kind and restaurant is shown in Table 2.

In terms of the comparison of the bacterial contamination of Ground-meat kebab among investigated restaurants, the highest and lowest amount of contamination in the samples was respectively related to B restaurant with average of 1.87x104 and C restaurant with the average of 1.13x104. Regarding Bakhtiari kebab and fish, the highest bacterial contamination was observed respectively in D restaurant with the average of 7.92x103 and A with the average of 9.67x102. But regarding salad, the highest and lowest level of contamination was found respectively in A and C restaurants with the average of 3.41 x 105 and 2.87 x 104 in each gram (Diagram 1).

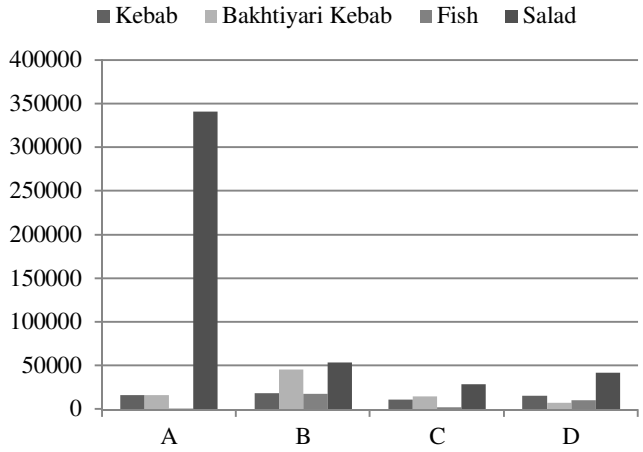

Diagram 1- The comparison of the average of total bacterial count in ready-to-use foods and salads in terms of restaurants tested

Regarding total bacterial count, the highest contamination was among the samples of B restaurant and regarding contamination to Coliform, *Escherichia coli* and *Staphylococcus aureus*, the highest contamination was observed in A restaurant. The difference between Coliform counts in Ground-meat kebab samples of this restaurant with 3 other restaurants was statistically significant ($p<0.05$). This was true in contamination to *Escherichia coli* and the highest level of contamination was associated to A restaurant. In the way that of 5 cases contamination to *Escherichia coli*, 4 cases (80% positive cases) were related to A restaurant (3 samples of salad and 1 sample of Ground-meat kebab).

Discussion

The results of this study showed that among the ready-to-use foods tested, salad and fish samples are respectively highest and lowest bacterial infection and comparing to the standard total bacterial count in salad (10^6 per gram) and cooked protein foods (10^5 per gram), it can be said that total bacterial count in all samples tested is at standard level. Higher contamination of salad and then Ground-meat kebab comparing to other kind of ready-to-use foods is justifiable, firstly, because the possibility of manipulation of food by the kitchen staff is more, secondly, there is the possibility of the contamination of kitchen appliances and equipment (knives, trays, meat grinder, etc.) used for salad and Ground-meat kebab preparation. Meanwhile heat is not used in preparing salad. In ground-meat kebab, minced meat is used which has higher load of bacterial comparing to other meats and it is cooked in most centers in traditional manner so it is possible that sufficient heat does not reach to deep parts of the kebab.

In studies conducted in other countries, hygiene and infection status of salad samples and foods prepared from minced meat to pathogenic bacteria has been studied. *Aycicek* et al. studied the infection of ready-to-use foods and salads to *Staphylococcus aureus* in restaurants of military centers at Ankara, Turkey and they tested total amount of 512 samples from different kind of salads, pizza and different meat foods preparing traditionally including Donner kebab. 48 samples (9.4%) were infected to positive *Staphylococcus aureus* coagulase with the average of 2.2×101 to 4.3×10^1 per gram and in salads (Russian salad and vegetable salad) and a kind of meat food (called meatball) which in most of them hand is used for their preparation, contamination to *Staphylococcus aureus* was significantly higher than other samples [27]. In the study by *Meldrum* et al. in U.K on 1213 vegetable salad samples and 1208 sauce samples used in salad preparation it is showed that 4.7% of salad samples and 5% of sauce samples have not acceptable microbial quality and the presence of *Staphylococcus aureus* and *Escherichia coli* is confirmed ($\geq10^2$ per gram). Also, a sample of salad was contaminated with salmonella [28]. In our study, infection to *Escherichia coli* in 4.16% of the salad samples is confirmed. In the *Sagoo* et al.' study infection to *Escherichia coli* in 3% of the salad samples is confirmed which is consistence with our study [29]. Among the four restaurants studied, the highest microbial load of foods was related to the samples of A and B restaurants respectively that indicates the insufficient

consideration to the health standards by two restaurants. This is probably due to lack of personal hygiene by staff, infection of the raw material or secondary contamination. For example, according to the studies, many workers employed in the food industry and restaurants have bacterial contamination [9, 1].

In this study, all samples tested were contaminated to Coliform higher than the standard level and maximum and minimum contamination were determined in salad and fish samples respectively. About the low contamination of fish samples it can be said that in general, the bacterial contamination is less in fish than chicken than minced meat and the less work is done for its preparation in cooking centers. The high contamination of salad and food samples to *Staphylococcus aureus* and Coliform (especially *Escherichia coli*) is considered as a serious alarm for health officials. Regarding *Staphylococcus aureus* probable cause of high rate of contamination of food samples tested is that this bacterium in raw materials is not good rival for other bacteria but in cooked foods that other microorganisms are omitted, grow easily and make contamination.

Absence of Salmonella in 288 food samples tested indicated lack of contamination of raw material and "cross contamination". In our study the highest contamination of Ground-meat kebab and salad is observed in (A and B) restaurants where the distance between the processing, cooking and eating is more than other restaurants that its probable cause can be higher number of customers and prolonged distance between the time of preparing and consumption of the food.

This is due to the higher number of customers, raw materials required for preparing Ground-meat kebab and also since different kind of salads are prepared several hours before, thus the likelihood of contamination is increased. According to the experts of health of the food, if the interval between food production and consumption takes more than 2 hours, the risk of food contamination is increased [26].

One of the limitations of this study was the lack of research on other foods due to lack of adequate funding. It is worth mentioning that in restaurants studied, other foods such as pizza, cooked rice and stew, Kashk-e-badenjan (fried eggplants) and so on are served and the selection of the foods such as Ground-meat kebab, Bakhtyari, fish and salad has been due to their higher risk and consumption. Also it was hard to obtain the satisfaction and agreement of managers of restaurants and kitchens.

The results of this study can be used by the managers of restaurants studied and health officials of military centers because the managers of the restaurants can reduce bacterial contamination in foods and salads using offered proposals. Study of Implementation of HACCP system and other standard certifications can be very effective in this regard. Further, complementary studies can determine the possible sources of contamination that in this regard, investigation on bacterial contamination in kitchen environment and dining hall, dishes and equipment used in processing and cooking and personal hygiene of staff is recommended.

Conclusion

Some foods and salads offering in the restaurants surveyed, have bacterial infection higher than standard and further study to determine contamination sources in kitchen environment and dining hall, dishes and equipment used in processing and cooking and implementing health education programs for staffs besides regular and periodic monitoring of these restaurants is necessary.

References

1- Razavilar V. Pathogenic bacteria in food. 3rd ed. Tehran: Tehran University Publication; 2010. [Persian]

2- Ryan CA. Massive outbreak of antimicrobial resistant salmonellosis traced to pasteurized milk. J Med Assos. 2002;278:389-95.

3- Henesy T, Brod G. A national outbreak of Salmonella enteritidis infections from ice cream. J Med. 2001;334:1281-6.

4- Tavakoli HR. Food microbiology and control of food production and distribution centers. 2nd ed. Tehran: Marz-e-Danesh Publication; 2008. [Persian]

5- FDA/CFSAN. Food-borne pathogens: Microorganisms and natural toxins. USA: International Medical Publication; 2008.

6- James MJ. Modern food microbiology. Mortazavi A, translator. Mashhad: Mashhad University Publication; 2007. [Persian]

7- Vanderzant C, Splittstoesser DF. Compendium of methods for the microbiological examination of foods. 3rd ed. Washington: American Public Health Association; 1992.

8- Roncesvalles G, Diez-Leturia M, Garcia-Jalon I. Food safety and the contract catering companies: Food handlers, facilities and HACCP evaluation. Food Control. 2011;22(12):206-12.

9- Chapman B, Eversley T, Fillion K, MacLaurin T, Powell D. Assessment of food safety practices of food service food handlers: Testing a communication intervention. J Food Pro. 2010;73(6):1101-7.

10- The Institute of Standards and Industrial Research of Iran Standards. Food microbial testing. Tehran: Institute of Standards and Industrial Research of Iran Standards; 2006. [Persian]

11- Kilic B. Current trends in traditional Turkish meat products and cuisine. Food Sci Technol. 2009;42(4):1581-9.

12- Lin CM, Fernando SY, Wei CI. Occurrence of Listeria monocytogenes, Salmonella spp, Escherichia coli and E. coli O157:H7 in vegetable salads. Food Control. 1996;7(3):135-40.
13- Soriano JM, Rico H, Molto JC, Manes J. Incidence of microbial flora in lettuce, meat and Spanish potato omelets from restaurants. Food Microbiol. 2001;18(2):159-63.
14- Ripabelli G, Sammarco ML, Fanelli I, Grasso GM. Occurrence of campylobacter, salmonella, vibrio, Y. enterocolitica, listeria and E. coli in fresh vegetables. J Food Hyg. 2002;118(2):137-47.
15- Fang J. Microbiological quality of ready-to-eat foods sold in Taiwan. Int J Food Microbiol. 2003;80(3):241-50.
16- Ghadge N, Kamat A. Assessment of microbiological quality of some raw salad vegetables from local market. Asian J Mic Bio Env Sci. 2004;6(4):729-34.
17- Meldrum RJ, Smith RM, Ellis P, Garside J. Microbiological quality of randomly selected ready-to-eat foods sampled between 2003 and 2005 in Wales. Int J Food Microbiol. 2006;108(3):397-400.
18- Froder H. Minimally processed vegetable salads: Microbial quality evaluation. J Food Prot. 2007;70(5):1277-80.
19- Bahk Gyung J, Todd E, Chong-Hae H, Deog-Hwan O, Sang-Do H. Exposure assessment for Bacillus cereus in ready-to-eat Kebab selling at stores. Food Control. 2007;18(6):682-8.
20- Abadias M, Usall J, Anguera M, Solsona C, Vinas I. Microbiological quality of fresh, minimally-processed fruit and vegetables and sprouts from retail establishments. Int J Food Microbiol. 2008;123(1-2):121-9.
21- Abougrain AK, Nahaisi M, Nuri SM, Mohamed M, Ghenghesh K. Parasitological contamination in salad vegetables in Tripoli-Libya. Food Control. 2010;2(21):760-2.
22- Ponniah J. Listeria monocytogenes in raw salad vegetables sold at retail level in Malaysia. Food Control. 2010;21(5):774-8.
23- Gormley FJ, Little CL, Grant KA, Pinna E, McLauchlin B. The microbiological safety of ready-to-eat specialty meats from markets and specialty food shops: A UK wide study with a focus on Salmonella and Listeria monocytogenes. Food Microbiol. 2010;27(4):243-9.
24- Christison CA, Lindsay D, Von Holy A. Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. Food Control. 2008;19(7):727-33.
25- Salek S. Microbial food intake of medical centers [dissertation]. Tehran: Shahid Beheshti University; 1999. [Persian]
26- Tavakoli HR, Riazipour M. Microbial quality of cooked meat foods in Tehran university's restaurants. Pak J Med Sci. 2008;24(4):595-9.
27- Aycicek H, Cakiroglu S, Stevenson TH. Incidence of S. aureus in ready-to-eat meals from military cafeterias in Ankara, Turkey. Food Control. 2005;16(6):531-4.
28- Meldrum RJ, Little CL, Sagoo S, Mithani V. Assessment of the microbiological safety of salad vegetables and sauces from kebab take-away restaurants in the United Kingdom. Food Microbiol. 2009;26(6):573-7.
29- Sagoo SK, Little CL, Mitchell RT. Microbiological quality of open ready-to-eat salad vegetables: Effectiveness of food hygiene training of management. J Food Prot. 2003;66(9):1581-6.