

Hygienic Aspects of Different Items of Salad Consumed in the local restaurants in Cairo.

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ABSTRACT

Food establishment could be a good source of spreading food born diseases. Some food products, such as salad, which don't require heating during their preparation, are potentially dangerous for the consumers. Therefore, good hygiene practice is very important for the preparation of salad. The aim of this study was to monitor the hygienic status maintained during the preparation of salads in the local restaurants in Cairo, Egypt. A total of 240 samples of different items of salads were collected randomly from several restaurants in Cairo. Microbiological analysis has been done for the detection of some emerging pathogens. It was found that *Salmonella spp.* was detected in 20%, 6.7%, 3.3%, 6.7%, 13% and 3.3% of samples from green salad, Tabola, Humus, Tehina, , Mayonnaise, Yoghurt respectively. While *Shigella* and *Listeria monocytogen* were detected only in Green salad and Tabola in the same percentage of 13% and 3.3% respectively. High *Enterobacteriaceae* and *Coliform* counts were detected in Green salad, Toboola and Cucumber at percentage (100%, 100% and 66.7%) and (100% , 90% and 16.7%) respectively. While the lowest percent of detection of *Escherichia coli* was in Green Salad and Cucumber at percentage 33% and 3.3% respectively. *Staphylococcus aureus* was isolated from five kinds of salads(Green salad, Toboola, Tehina, Bab Ganooch and Mayonize in percent ranged from 16.7% to 90%.

These results are an indication that the possibility of contaminated raw materials, un cleaned equipments and unhygienic food handlers are the main sources of contamination of salads. Inspection of food establishment should be concentrated on the way of preparing salads as well as on the equipments, raw material used and the hygienic condition of the establishment and the food handlers.

INTRODUCTION:

Ingredients for the preparation of salads could be from different food items. Green vegetables, olives, white cheese and mayonnaise are the main items generally used in the preparation of salads. Fresh products such as vegetables are not only at risk of getting contaminated while in the field, but also this may happen during the post harvest handling, processing and distribution (Beuchat and Ryu, 1997). Sources of post harvest contamination may be harvest equipment, wash and rinse water, transportation and cross contamination. On the other hand, at preharvest stage, feces, irrigation water and human handling could be sources of microbial contamination (Endley *et al.*, 2003)

The consumption of commercially prepared salads has been increasing during the past few years. Salad, in general, are neither heated to ensure the destruction of pathogen and spoilage organisms, nor exposed to any treatment such as freezing and additting to prolong the shelf life. Some studies have been done to assess the safety of such foods (Christiansen and King, 1971; Fowler and Clark, 1975; Fowler and Foster, 1976). It was reported that some food poisoning cases were registered due to consumption of contaminated salads (Meyer and Oxhoj, 1964; Peterson, 1964; Helmy *et al.*, 1980). However, the information about the microbial content of salad is very scarce in the Middle East region (Helmy *et al.*, 1980). In Egypt, as well as in other countries in the region, green salad is the most popular one. In addition to other types of salads such as Homus, Tehina, mayonnaise salad etc. No detailed studies, however, have been done in Egypt to assess the microbial characteristics of salads. Therefore, the aim of this study was to monitor the hygienic status maintained during the preparation of salads in the local restaurants in Cairo

MATERIALS AND METHODS

A total of 240 salad samples representing 30 samples of Green salad Taboola, Homus, Tehina, Mutable, Baba Ganooch, Mayonaie and cucumber with yoghurt were collected from different restaurants in sterile containers in Egypt and brought to the laboratory in ice boxes. All the samples were analyzed for the determination of pathogens expected in this kind of foods, such as *Enterobacteriaceae coliforms*, *E. coli*, *Salmonella* spp., *Staph. aureus*, *Shigella* spp. and *L. monocytogenes*.

Preparation of the tested samples was done by initial suspension and decimal dilution according to ISO 6887-2-(2001) and ISO 6887-1.(1999). Ten grams of sample were weighted into a sterile stomacher bag and 90 ml diluents (buffered peptone water) was added and blended for 1-2 min. Then, decimal dilution to 10^{-7} in buffer peptone water was made to perform enumeration of *Enterobacteriaceae*, *Coliform*, *Staph aureus* and *E.coli*. Enumeration of Enterobacteriaceae (37°C /24 hrs) was determined using crystal violet neutral red bile dextrose (VRBD), (ISO 7402,1993). The coliform group enumeration was done by poor plate method according to ISO 4832,(2005) on crystal violet neutral red bile lactose (VRBL) and incubated at 37°C for 48 hrs.

Escherichia coli enumeration was done by surface plate method according to NMKL No. 125 – (1996) modified on VRB-Mug incubated at 44.5°C for 24 hrs.

Staph. aurus enumeration was done by surface plate method according to ISO 6888 1998 on Baird parker agar (BP) and incubated at 37°C for 48 hrs.

Salmonella were tested according to ISO 6579 – (2002). Pre-enrichment, 25g sample was performed in 225 ml buffer peptone water and incubated at 37°C for 16 – 20 hr. Then, 1 ml and 0.1 ml was transferred into 10 ml in two selective enrichment broth Tirta Thionate (TTB) broth and Rappart – vassiliadis broth (RV) and incubated at 37°C and 41.5°C for 24 hrs, respectively. One loop from each selective enrichment was streaked on Hektole enteric agar, XLD and phenol red Brilliant green agar at 37°C for 24 hrs. Suspected colonies were subjected to biochemical identification on lysine decarboxylase, triple sugar iron agar and urea agar at 37°C for 24 hrs. Positive colony subjected to confirmation by using API 20 E system and serology antibody reaction.

Shigella spp. were tested according to ISO 21567 – (2002). Enrichment, 25g sample was performed in 225 ml GN Broth containing 0.5 µg /ml and incubated anaerobic at 41.5°C for 16 – 20 hr. One loop from enrichment broth was streaked on MacConkey agar Hektole enteric agar and XLD agar at 37°C for 24 hrs. Suspected colonies were subjected to biochemical identification on lysine decarboxylase, triple sugar iron agar and urea agar at 37°C for 24 hrs. Positive colony subjected to confirmation by using API 20 E system and serology antibody reaction.

Listeria monocytogenes was tested according to ISO 11290-1(1996). 25g of sample were added to 225 ml half frazier broth and incubated at 30°C for 24hr .0.1 ml from primary enrichment was transferred to 10.0 ml of frazier broth (secondary enrichment) and incubated at 37 °C for 48hr . Two loops from second enrichment were streaked on Oxford and Palcam agar at 37°C for 24 hr .Suspected colony subjected to confirmation with API listeria.

Quality control :

General guidelines on quality assurance for the preparation of culture media were applied in the laboratory according to ISO 11133-1 (2000) and ISO 11133-2 (2002).

RESULTS AND DISCUSSION

Prevention of contamination is the most efficient way to insure food safety and prevent food born illness. Thus, much effort should be done to protect food from contamination. Raw food stuffs, particularly vegetables grown close to the soil, may be contaminated with microorganisms.

The specific types of microorganisms predominant in freshly harvested vegetables are quite variable. Because these foods are subjected to different environmental conditions. These foods can be contaminated with different types of microorganisms. In general, however, the typical micro flora of freshly harvested vegetables could be gram

negative bacteria. The specific bacteria include *Enterobacter* spp and *Pseudomonas* spp. (Brackett, 1998; Skovgaard, 1984 and Zhao *et al.*, 1997). Enterobacteriaceae bacteria that are found in the human or animal intestinal tract, such as *Salmonella* and *Shigella*. Enterobacteriaceae are useful indicators of hygienic condition of foods.

Among 240 samples of different types of salads analysed 15 samples were found to be contaminated with *Salmonella* and 5 samples were contaminated with both *Shigella* and *Listeria monocytogen*. Table (1) showed that *Salmonella* spp. was detected in 20%, 6.7%, 3.3%, 6.7%, 13% and 3.3% in samples from green salad, Tabola, Humus, Tehina, Mayonnaise, Yoghurt respectively. While *Shigella* and *Listeria monocytogen* were detected only in Green salad and Tabola in the same percentage of 13% and 3.3% respectively. Beuchat (1996) stated that bacterial pathogens may contaminate vegetables at any point throughout the production system. Potential pre-harvest sources of contamination include soil, feces, irrigation water, water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals and human handling.

All of the Green salad samples and, Taboola samples were contaminated with *Enterobacteriaceae* at counts ranging from (7.5×10^3 to 1.7×10^4 cfu/g) and (6.0×10^2 to 1.5×10^3 cfu/g) respectively. While the frequencies of incidence of *Enterobacteriaceae* in Homus, Tehina, Baba ganooch, mayonaize, and cucumber were 6.7%, 16.7%, 33.3% and 66.7% respectively. On the other hand the *Enterobacteriaceae* was not detected in Yoghort Table (2).

Coliform not detected (< 10 cfu/g) in both kinds of salad Homus and Yoghort. While the frequency of incidence of *Coliform* in green salad, Tabola, Tehina, Baba Ganooch, Mayonaize, and Cucumber were 100%, 90%, 2.7%, 6.7%, 6.7% and 16.7% respectively.

Helmy *et al.* (1980) have reported the detection of *Coliforms* in 19 out of 20 samples of Tehina salad. They showed counts ranging from 1.4×10^4 to 4.0×10^5 cells/g. In the present case, the *Coliform* counts in Tehina was detected in one sample out of thirty sample. It was considered relatively low showing count 1.5×10^2 cfu/g. However, this is indicative of unhygienic handling during the preparation.

Coliform counts in Green salad were higher than that encountered in Tehina, the range being 5.0×10^2 to 1.1×10^3 cfu/g. Besides these, two of the samples also showed the presence of *E. coli* (1.0×10^2 cfu/g and 9.0×10^2 cfu/g) and twenty seven samples had coagulase positive *S. aureus* ranged from (5.0×10^2 cfu/g to 5.0×10^3 cfu/g). These high counts of Coliforms and the presence of *E. coli* and *S. aureus* might have originated either from

the raw materials or from the handlers. Prokopowich and Blank (1991) reported a coagulase positive *S. aureus* contamination rate of 24% in the vegetable sprouts and seeds they surveyed. However, it is questionable whether this organism would be able to grow on these produces in large numbers and produce enterotoxin leading to public health risk. Growth of *S. aureus* on salad bar vegetables to a concentration of about 10^5 cfu/g, but without the production of enterotoxin has been reported by Gourama *et al.* (1991). Homus, Tehina, Baba Ganouch, Mayonaize, and Yoghort were totally free from any E Coli. While the incidence of E Coli in Green salad, Taboola and Cucumber were 33%, 6.7% and 3.3% respectively. The frequencies of incidence of Staph. aureus in Green salad, Taboola, Tehina, Baba Ganouch and Mayonaize were 90%, 66%, 33%, 16.7% and 23.3% respectively.

Table 1: Prevalence of pathogenic micro organisms in different kind of salads

	Sallmonella		Shigella		Listeria	
	prevalence	%	prevalence	%	prevalence	%
Green salad	6(30)	20%	(30)	13%	4(30)	13%
Tabola	1(30)	6.7%	1(30)	3.3%	1(30)	3.3%
Homus	1(30)	3.3%	0(30)	0%	0(30)	0%
Tehin	2(30)	6.7%	0(30)	0%	0(30)	0%
Bab Ganoch	0(30)	0%	0(30)	0%	0(30)	0%
Mayonnaise	4 (30)	13%	0(30)	0%	0(30)	0%
Yoghort	1(30)	3.3%	0(30)	0%	0(30)	0%
Cucumber	0(30)	0%	0(30)	0%	0(30)	0%

The high counts of Enterbacteriaceae, coliform, the presence of *E. coli* and *S. aureus* might have originated either from the raw materials or from the handlers. Prokopowich and Blank (1991) reported a coagulase positive *S. aureus* contamination rate of 24% in the vegetable sprouts and seeds they surveyed. However, it is questionable whether this organism would be able to grow on these produces in large numbers and produce enterotoxin leading to public health risk. Growth of *S. aureus* on salad bar vegetables to a concentration of about 10^5 cfu/g, but without the production of enterotoxin has been reported by Gourama *et al.* (1991).

A number of earlier studies have shown that the pathogenic bacteria not only survive but can also grow on vegetables under congenial conditions (Abdoul-Raouf *et al.*, 1993; Farber *et al.*, 1998; Gourama *et al.*, 1991; Thunberg *et al.*, 2002; Endley *et al.*, 2003). *Salmonella* has been detected in lettuce, cauliflower, sprouts, mustard cress, spinach etc. (Beuchat, 1995; Blaser *et al.*; 1981, Mahon *et al.*, 1997; Ooi *et al.*, 1997). *E. coli* 1057:H7 has been detected by Beuchat (1995) in sprouts, cabbage, lettuce and cilantro. *S. aureus* including enterotoxin producing strains have been detected in a number of fresh produces such as alfalfa sprouts, broccoli, broccoli sprouts, cauliflower, celery, lettuce, and mung bean sprouts (Thunberg *et al.*, 2002). They also have reported the presence of a variety of *Listeria* spp. including *L. monocytogenes* in these produces.

Also it has been reported that food born outbreaks were linked to the consumption of raw vegetables, epidemiological investigation identified manure as the source of contamination: *L. monocytogenes* on cabbage in Canada, and *Salmonella* and *E. coli* on apples used in making apple juice in U.S. (Nguyen-the & Carlin 2000 and Tauxe *et al.*, 1997).

Ready-to-eat vegetables which form the major ingredients of green salads and other ingredients such as sesame seed paste (Tehina), chick pea paste (Homus) etc. are subjected to minimal or no processing before consumption. Hence, these produces are prone to get contaminated with pathogenic microorganisms, which can occur at any stage from farm to the dining table. Contaminated raw materials, uncleaned equipments and unhygienic food handlers are main sources of contamination of salads. Inspection of food establishment should be concentrated on the way of preparing salads as well as on the equipments, raw material used and the hygienic condition of the establishment and the food handlers.

Table 2 incidence of different microorganism (cfu/g) in different kind of salads

Kind Of salad	Enterobactria cfu/g			coliform cfu/g			E.coli cfu/g			staph aures cfu/g		
	N.D	Mean	range	N.D	Mean	range	N.D	Mean	range	N.D	Mean	range
-Green salad	---	15000	(7500-17000)	---	8000	500-1100	20	500	100-900	3	3000	500-5000
		100%			100%		66%	33%		10%	90%	
-Toboola	---	12000	(600-1500)	3	6500	(300-900)	28	500	(400-600)	10	2500	300-4000
		100%		10%	90%		93.3%	6.7%		33.3%	66%	
-Homus	28	200	150-250	30	---	---	30	---	---	30	---	---
	93.3%	6.7%		100%			100%			100%		
-Tehina	25	500	200-700	29	150	150	30	---	---	20	1500	500-2000
	83.3%	16.7%		97.7%			100%			66.7%	33.3%	
-Baba Ganooch	23	300	100-500	28	150	30	30	---	---	25	500	100-700
	76.7%	23		93.3%	6.7%		100%			83.3%	16.7%	
-Mayonaie	20	500	100-900	28	100	50-150	30	---	---	23	100	200-1500
	66.7%	33.3%		93.3%	6.7%		100%			76.7%	23.3%	
-Yoghort	30	---	---	30	---	---	30	---	---	30	---	---
	100%	0%		100%			100%			100%		
-Green salad	10	1000	500-2000	25	800	300-1100	29	150	150	30	---	---
	33.3%	66.7%		83.3	16.7%		97.7%	3.3%		100%		

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الملخص العربي

الشروط الصحية لبعض انواع السلطة المتواجدة من مطاعم القاهرة

فؤاد الطحان

مركز البحوث الزراعية - المعمل المركز وتحليل متبقيات المبيدات والعناصر الثقيلة في الاغذية - دقى - جيزة

خطوات تأمين الطعام من الممكن ان تكون من اهم المصادر لانتشار الأمراض . بعض مصادر الطعام مثل السلاطة التي لا تحتاج إلى تسخين أثناء التحضير تعتبر ذات خطورة قوية على المستهلك . لذلك يجب توفير الاشتراطات الصحية في تحضير السلاطة يعتبر امر هام . الهدف من هذه الدراسة هو متابعه الخطوات الصحية التي تتم أثناء تحضير السلاطة في اطعمة الوجبات السريعة . مجموعه من ٢٤٠ عينة من مختلف عناصر السلاطة التي تم تجميعها عشوائيا من مطاعم عديدة في القاهرة .

وقد تم عمل تحليل ميكروبي لاكتشاف بعض انواع البكتريا الممرضة . وقد وجد ان بكتريا السالمونيلا اكتشف في ٢٠% ، ٦,٧% ، ٣,٣% ، ٦,٧% ، ١٣% ، ٣,٣% من عينات السلاطة الخضراء ، التبوله ، حمص ، مايونيز والزبادى بالتتابع . اما بكتريا الشيجيلا و الليستيريا فقد اكتشف فقط فى السلاطة الخضراء والتبوله بنفس النسب ١٣% ، ٣,٣% بالتتابع .

وقد وجد في السلاطة الخضراء ، التبوله والخيار أعداد كبيرة من مجموعة الكوليفورم و الانتيرو بكتيريا بنسب (٦٦,٧% ، ١٠٠% ، ١٠٠%) و (١٦,٧% ، ٩% ، ١٠٠%) بالتتابع . اما اقل نسبه اكتشفت فى السلاطة كانت عنصر الايشريشياكولى وكانت فى السلاطة الخضراء والخيار بنسب ٣٣% ، ٣,٣% بالتتابع .

وقد تم عزل بكتيريا العنقود الذهبى من ٥ عينات من أصناف السلاطة وهى السلاطة الخضراء ، التبوله ، الطحينه ، بابا غنوج والمايونيز بنسب تتراوح بين ١٦,٧% ، ٩% . هذه النتائج توضح ان احتمالات تلوث المواد الخام ، وعدم نظافة الادوات وعدم تطبيق الاشتراطات الصحية للأشخاص المتواجدين داخل طرق تأسيس الطعام يعتبر هو المصدر الرئيسى لتلوث السلاطة . متابعه طرق تأسيس الطعام يجب ان تركز على طرق تحضير السلاطة وايضا الادوات والمواد الخام المستخدمه والجو الصحى والأشخاص داخل خطوط التأسيس للطعام .