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Microbial analysis of vegetable salad served in restaurants of Hail city, Saudi Arabia

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ABSTRACT

It is well known that fresh vegetables play significant role in your healthy diet. Consumption of raw vegetables salad represented an important mean of transmission of various infectious diseases which is accountable for global public health problem. It plays a significant role in human morbidity, mortality and economic loss. The aim of the present study was to determine microbial and parasitic contamination of ready-to-eat vegetables salad served in restaurants of Hail city, KSA. A total of 25 different ready-to-eat vegetables salads were collected aseptically from different restaurants of Hail city, Saudi Arabia; and were analyzed for total heterotrophs and coliforms along with parasites. The highest number of viable count was found in sample number R-2 (4.91 log CFU/g) and least microbial loads (2.25 log CFU/g) were observed in sample number R-9. A significant microbial load was recorded in the samples even after third washing. Intestinal parasites were also detected in 16% of unwashed samples and not in standard washed samples. There was significant difference between ready-to-eat vegetable salad and salad after three successive washing. This study revealed the potential hazard of ready-to-eat salads. These findings may have important implications for global food safety and emphasize the importance of raw vegetables salad in threatening public health by transmission of microbes and parasites to humans in Hail, KSA. These results indicate the need of implementing quality programs in the production chain of ready-to-eat vegetable salad to improve microbiological safety.

Key words: *Vegetable salad, washing procedure, heterotrophs, coliforms, parasites, restaurants, Saudi Arabia*

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1. INTRODUCTION

Green vegetables are always been an important food item for human consumption. Raw vegetables are great source of vitamins, dietary fiber and minerals; and their regular consumption is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers (Van Duyn and Pivonka, 2000). Green salad is a daily habit of more than 50% individuals in Saudi Arabia. The most common salad vegetables used in Saudi Arabia are Parsley (*Petroselinum crispum*), Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) and Gargeer (*Eruca sativa*). Parsley is an annual herb indigenous to the Mediterranean region, but now cultivated worldwide. Parsley is an excellent source of vitamin C and K along with vitamin A, folate and iron. Romaine lettuce is also a great source of vitamin C. Aside from boosting your immune system, vitamin C helps keep your skin, bones, and teeth strong. These beneficial effects associated with vitamin C are linked to its strong antioxidant properties as well as its role in collagen

synthesis. Gargeer, also known as Arugula is one of the nutritious green-leafy vegetable of Mediterranean origin. It is a small and low growing annual herb with rich source of certain phytochemicals such as indoles, thiocyanates, sulforaphane, and isothiocyanates. Together, these compounds have been found to counter carcinogenic effects of estrogen and thus help protect against prostate, breast, cervical, colon, and ovarian cancers. Microbial threats are considered as one of the biggest extortions to food safety (Elhariry, 2011). Bacteria are considered as the most common food poisoning agents. More than 90% of the cases of food poisoning each year are caused by *Staphylococcus aureus, Salmonella* spp, *Clostridium perfringens, Campylobacter* spp, *Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus,* and Entero-pathogenic *Escherichia coli* (Khiyami et al., 2011). Further tendency of people for eating raw or slightly cooked vegetables to protect heat-labile nutrients may increase the risk of foodborne infections. Every year, millions of individuals become ill from food borne diseases and in that salads vegetables may be sources of pathogen transmission (KACST, 1993; Jones et al., 2008). In recent years, the occurrence of antibiotic resistant strains of a number of pathogenic bacteria has emerged as another health concern all over the world (Graziani et al., 2004). Several outbreaks of intestinal parasitic infections epidemiologically associated with raw vegetables have been reported from developed and developing countries (Mintz et al., 1993; Poorna and Randhir, 2001). Although raw vegetables (Salad) are important part of human diet, there is more chance of microbial infection in human being through consumption of salad, contaminated with various microorganisms. The consumption of raw vegetables without proper washing is an important route in the transmission of parasitic diseases also (Slifko et al., 2000). Ready-to-eat salads are considered as a high-risk food because they do not require any heating or standard washing prior to consumption. The microbial infection may cause a serious health problem. No previous studies have been conducted to evaluate the prevalence of microbial contamination of minimally processed ready-to-eat vegetables salad in Saudi Arabia. In the context of growing awareness on microbial quality of fresh and raw vegetables in salads, the present study was conducted to enumerate various microorganisms in commonly used vegetables salad served at different restaurants of Hail city, Saudi Arabia.

2. Materials and Methods

The present study was carried out in Hail city, which is the capital of Hail province, Saudi Arabia, during the period of August 2016 - December 2016. The study included ready-to-eat vegetable salad samples, comprised of three leafy vegetables *viz.* Lettuce, Parsley and Gargeer, from randomly selected restaurants of the city.

2.1 Sample collection and processing

A total of 25 ready-to-eat vegetable salad samples were collected randomly from various restaurants of Hail city and was carried out to the laboratory in autoclaved poly bags. All the samples were obtained as fresh in the original package and subjected to analysis within 2-4 hours of sample collection.

2.2 Microbial analysis

Integrated samples of vegetables salads (10g each) were washed with 100 ml of sterile distilled water separately and the water drained after washing was collected in a sterile bottle.

Washing was carried out thrice and water was collected every time in separate bottles. The collected water samples were used for quantitative estimation of heterotrophs and coliforms. The total number of heterotroph and coliforms was isolated and enumerated on Nutrient agar and Mac-Conky agar media, respectively employing serial dilution technique according to the standard method of Nakayama (1981). Diluted suspensions (0.5 ml) was spread over the solid nutrient agar plates and incubated at 37±1ºC for three to five days and the total numbers of heterotroph and

coliforms was determined as a colony forming unit per ml (CFU ml^{-1}) of sample. Total number of microbes per gram of salad was calculated accordingly. On the basis of their morphological differences organisms was isolated for further studies. Pure cultures were stored in agar stabs at -20ºC for further studies.

2.3 Detection of intestinal parasites

The unwashed vegetable salad samples were examined for the presence of parasites. A weighed sample (10 g) was placed into a sterile bottle and washed with 100 ml physiological saline solution (0.9% NaCl). The washing water left overnight for sedimentation. Afterward, the top water was discarded and the remaining washing water was centrifuged at 2000Xg for 15 min. The supernatant was removed, the residue carefully collected in a test tube and mixed with a drop of Lugol's Iodine solution. A drop of mixture (residue) was transferred on the center of a grease-free clean glass slide and covered gently with a clean cover slip. The preparation was examined under a light microscope using X10 and X40 objectives for detection of parasites. The process was systematically repeated until the mixture in each test tube was exhausted (Downes and Ito, 2001). The efficiency of washing procedures to remove parasites from ready-to-eat vegetables salads is also evaluated.

2.4 Statistical analysis

Statistical analyses were carried out using SPSS software version 9.0 for windows (SPSS Inc., Chicago, IL, USA) to compare the rate of contamination of vegetable salad among different restaurants. The differences will be considered significant at $P < 0.05$.

3. Results and discussion

The results of microbial analysis of ready-to-eat packed vegetable salad from different restaurant of Hail city are presented in table 1. The results shows high populations of aerobic microbes present in minimally processed vegetable salad samples (Fig. 1), suggesting lack of good hygienic practices during the processing and packaging of vegetable salads at different restaurants. The total heterotrophs count (log CFU per gram) varies from 4.91 to 2.25. However, the coliforms count varies from 4.47 to 1.8. This difference in the microbial contamination could be due to the mishandling of salad and disregard to hygienic measures by worker in each restaurants which may introduce contaminants and pathogens to food throughout the chain of production, processing, storage and preparation. Also, the total number of bacteria was enumerated after each washing in order to assay the reduction rate of bacterial load on vegetable salad after three successive washings. The data on colony-forming units of both heterotroph and coliforms showed near about 20-80 % reduction after each successive washing. Even though after third washing the significant load of heterotrophs (2.07 log CFU/g) and coliforms (1.1 log CFU/g) was recorded. Although, further processing like addition of some preservatives to vegetables may reduce the microbial load to some extent, a considerable amount of microorganisms could enter the human body. However, upon visual inspection, all the samples analyzed in this work appeared to be suitable for consumption.

Table 1. Bacterial counts and parasitic contamination in the minimally processed ready-to-eat vegetable salad samples (values are average of 3 replications)

Salad samples	Heterotrophs (log CFU/g[a])			Coliforms (log CFU/g[a])			Parasites	
	1st washing	2nd washing	3rd washing	1st washing	2nd washing	3rd washing	Unwashed	Washed
R 1	3.90	3.72	3.50	3.54	3.15	2.95	-	-

R 2	4.91	4.50	4.44	4.47	4.30	4.15	-	-
R 3	4.70	4.20	3.85	4.55	3.60	3.25	+	-
R 4	2.94	2.44	2.20	3.51	3.04	2.89	ND	ND
R 5	4.12	5.05	ND	4.12	3.36	3.28	-	-
R 6	4.82	4.40	4.31	4.40	4.25	4.08	-	-
R 7	3.90	3.71	3.69	3.85	3.68	3.11	-	-
R 8	3.97	3.90	3	4	3.80	3.59	-	-
R 9	2.25	2.20	2.07	1.80	1.35	1.1	-	-
R 10	3.61	3.42	3.30	3.32	2.76	2.60	+	-
R 11	4.80	4.01	3.20	4.04	3.20	2.10	-	-
R 12	4.01	3.45	2.70	4.10	3.30	2.45	+	-
R 13	4.60	3.20	2.85	4.20	3.30	2.25	-	-
R 14	3.94	2.86	2.50	3.90	3	ND	-	-
R 15	4.32	3.60	2.70	4.12	3.40	2.20	-	-
R 16	4.02	3.10	3.31	3.40	3.05	2.18	+	-
R 17	4.20	3.12	2.69	3.60	2.68	2	-	-
R 18	3.50	2.90	2.40	4.10	3.80	2.20	-	-
R 19	3.25	3.20	2.80	2.45	2.04	1.90	-	-
R 20	4.61	3.70	3	3.40	3.10	2.30	-	-
R 21	3.90	3.03	ND	3.60	2.75	1.60	-	-
R 22	3.65	2.70	2.40	3.90	3	2.40	ND	ND
R 23	3.92	3.50	3.45	4.30	3.30	2.12	-	-
R 24	4.60	4	3.20	3.55	2.60	1.25	ND	ND
R 25	3.80	2.95	2.35	3.60	2.25	1.45	-	-

[a]Colony Forming Units per gram of salad, ND=Not done

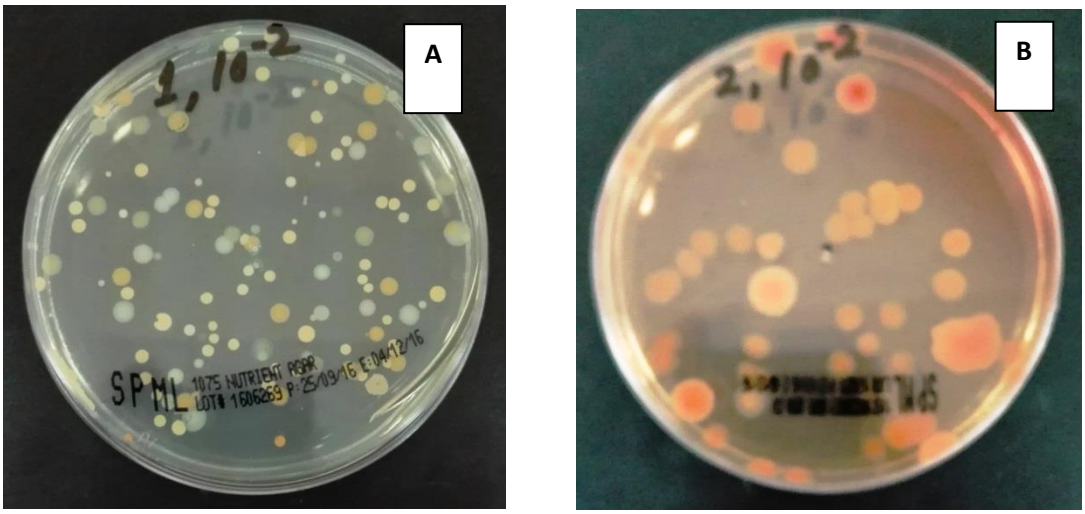

Fig 1. Microbial contamination in ready-to-eat vegetables salad (A-Nutrient agar, B-MaKoncky agar)

According to our knowledge, there is no study on the ready-to-eat vegetable salads served in restaurant of Hail, Saudi Arabia. However, studies on leafy vegetable consumed in Hail are reported by some workers (Al-holy et al.,

2013; Abu-rayyan et al., 2016). Other studies from different countries are also reported occurrence of bacterial populations on ready-to-use vegetable (Fenlon et al., 1996; Doris and Seah, 1995). Soriano and coworkers (2000) reported the isolation of *E. coli* from restaurant prepared lettuce. *Salmonella* species has been isolated from a range of vegetables (Garcia-Villanova et al., 1987) and S*almonella* food poisoning out breaks have been associated with the consumption of plant food such as tomatoes (Wood et al., 1991). Hesteriosis outbreaks have previously been epidemiologically associated with the consumption of raw salad vegetables (Ho et al., 1986). A recent foodborne disease outbreak during 2011 in Europe and Germany was mainly due to contamination of salads by harmful strain of *E. coli O104:H4* (WHO, 2011). *Salmonella* and *Staphylococcus aureus* were the other major pathogens found in salads. Also another study conducted in Dehradun, India, has shown that different types of salads were contaminated with *Salmonella*, *E. coli*, *S. aureus*, and *P. aeruginosa* (Dheeraj et al., 2012). The contamination of *Salmonella* spp. in vegetables salad may be due to washing of vegetables with contaminated water and handling of vegetables by infected workers and vendors in the market place which helps spread pathogenic microorganisms. Furthermore, preference for eating raw vegetables to protect heat labile nutrients may increase the risk of foodborne infections.

The results also highlight the potential of vegetables salad in transmission of intestinal parasites to human. A very few studies have been conducted in Saudi Arabia to evaluate the degree of parasitic contamination in ready-to-eat vegetable salads. This is the first research carried out to determine parasitic impurity along with microbial contamination in ready-to-eat vegetable salads served at restaurants of Hail, Saudi Arabia. The results of parasitic contamination in unwashed and washed vegetable salad samples are shown in Table 1. Along with bacterial load as discussed above, intestinal parasites were also detected in 16% (4/25) of the unwashed salad samples (Fig. 2). In agreement with the present study, a high prevalence of parasitic contamination in vegetables has also been reported in the other study of Hail region (Al-holy et al., 2013; Abu-rayyan et al., 2016). Our study revealed that proper washing of sample significantly reduced the rate of parasitic contamination comparing to unwashed samples. However, elimination of parasites by using traditional washing procedure (quick washing without disinfecting) is not guaranteed. In contrast, no parasite was found in the salad samples washed with standard procedure (Table 1) which emphasize the use of standard washing procedure (proper washing and disinfecting) before consumption of vegetables salad instead of traditional method (quick washing without disinfecting). Other researchers also suggested that proper washing of salad vegetables prior to consumption and packing can prevent transmission of the parasites to human (Avcioglu et al., 2011; Kozan et al., 2005; Shahnazi and Jafari-Sabet, 2010).

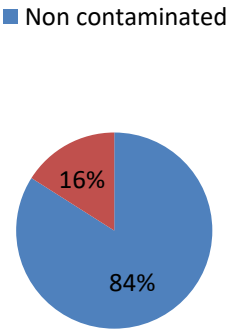

Fig. 2. Percentage of contaminated and non-contaminated ready-to-eat vegetable salad samples at restaurants of Hail, Saudi Arabia

The use of untreated wastewater for irrigation of vegetable fields could be a reason for parasitic contamination. The organisms in salads indicate possible fecal contamination of green vegetables and poor hygienic processing practices (Tambekar and Mundhada, 2006). Handling of vegetables with contaminated hands and utensils, insufficient washing of vegetables and use of untreated waste water for washing purposes lead to the unhygienic status of salads (Greig et al., 2007; Todd et al., 2007a; 2007b). Although we did not examine samples of irrigation water used for salad vegetables, our findings indicate that the vegetables would be irrigated with sewage water. There is a need to inform the public in general and restaurant owners in particular of importance of properly washing/disinfecting raw vegetables before consumption and packing for ready-to-eat salad. The local health and environmental authorities should educate the public on the health hazards of fresh salad vegetables and the importance of washing and disinfecting them before consumption.

4. Conclusion

In conclusion, this study revealed the potential hazard of minimally processed ready-to-eat vegetable salads. The salads may cause infections in humans due to presence of heterotrophs, coliforms and associated parasites. Although these microorganisms can be part of the epiphytic flora of the vegetables used in salads, their proliferation is a reflection of poor hygienic practices by the food handlers. Therefore, there is a need to educate the public for the risk involved in the use of contaminated vegetable salad and the food processors should be educated about the path of contamination and need to improve hygienic practices during preparing the salads. A simple thorough washing of vegetables with safe running water may reduce the risk of microbiological hazards as proved by further frequent washing. These findings may have important implications for global food safety and emphasize the importance of ready-to-eat vegetables in threatening public health by transmission of microbes. Media programs should inform the consumers about potential health consequences due to consumption of ready-to-eat vegetables salad and the importance of proper washing and disinfecting of green vegetables before consumption.

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