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Food borne pathogen contamination in minimally processed vegetable salads in Riyadh, Saudi Arabia

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The increasing demand for fresh fruits and vegetables and for convenience foods is causing an expansion of the market share for minimally processed vegetables in different areas. Riyadh, the capital city of Saudi Arabia witness flourishing of hotel and food industry and eating green salad is a daily habit of about 50% individuals in the country. Hence microbial quality of minimally processed vegetable salads (Tabbouleh, Fattoush, Hummus, Mutabbel and Caesar) being served in restaurants and homes in Riyadh were evaluated to ascertain that they are safe for human consumption and are free from potential food borne pathogens. The samples were assessed for the presence of total aerobic bacterial plate count, total coliforms, *Escherichia coli*, *Salmonella*, and *Shigella*. The total aerobic plate count for salad prepared in the restaurants was around $2 - 4.5 \times 10^5$ CFU/g, however, in homemade salads the count was $2-8 \times 10^4$ CFU/g. The total coliform counts in restaurants salad were around $2-8 \times 10^4$ CFU/g as compared to $2-4.8 \times 10^3$ CFU/g of homemade salads. All salads, except Caesar, recorded *E. coli* and *Enterobacter aerogenes*, while *Shigella* sp and *Salmonella* sp were present in few samples. The results of the present study warrant an urgent need to have strict control measures to eliminate food borne pathogen contamination. This is the first report on microbial quality of the said minimally processed vegetable salads in Saudi Arabia.

Key words: Saudi Arabia, vegetable salads, fruits, food borne pathogen contamination.

INTRODUCTION

Microbial hazards continue to be one of the biggest threats to food safety (Al-Binali et al., 2006; Simon-Sarkadi, 2007; Elhariry, 2011). The increasing demand for fresh fruits and vegetables and for convenience foods is causing an expansion of the market share for minimally processed vegetables. Bacteria are the most common food poisoning agents. More than 90 percent 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*. Total

coliform counts (TC) and *E. coli* are used as hygiene-quality parameters. Thus, the presence of *E. coli* in foods is an indicator of direct or indirect fecal contamination. It is also an indicator of the possible presence of enteric pathogens (Gonzalez et al., 2003; Sher et al., 2010a). Each year, millions of individuals become ill from food borne diseases and those salads can be sources of pathogen transmission (KACST, 1993; Jones et al., 2008). Pathogens isolated from several kinds of salads include *S. aureus*, *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Salmonella typhi*, *Serratia* spp., *Providencia* spp. *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, and *Shigella sonnei* (Poorna and Randhir, 2001; Johannessen et al., 2002; Warren et al., 2007; Wright et al., 2009; Xanthopoulos et al., 2009). In recent years, the occurrence of antibiotic resistant strains of a number of pathogenic bacteria including *Salmonella*,

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E. coli, *Enterobacter* and *P. aeruginosa* has emerged as another health concern all over the world (Poorna and Randhir, 2001; Busani et al., 2004; Graziani et al., 2004).

Earlier investigators proved that vegetables were contaminated with microorganisms when they were irrigated with sewage water and when the soil was fertilized with manure (Priepke et al., 1976; Rosas et al., 1984). While salmonellae have been associated with animal products including poultry, meat, eggs and manure (Wagner and McLaughlin, 1986), their significance as vegetable borne pathogen widely distributed in nature, was ascertained since they were isolated from soil, water, sewage, silage, and animals (Brackett, 1988). Thus it might contaminate fresh vegetables at their source or subsequently during handling and marketing (Geldreich and Border, 1971). Further use of treated waste water for irrigation, poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensils also contribute to contamination (Ibrahim, 1996; Hussain and Al-Saati, 1999; Poorna and Randhir, 2001).

Among the more common pathogenic microorganisms that can be transmitted to humans by these products are *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella*. Several outbreaks of human gastro-enteritis have been linked to the consumption of contaminated fresh vegetables, fruits and sprouts (Poorna and Randhir, 2001). There is a growing market in the sale of pre-packed ready-to-eat salad vegetables which are stored under refrigerated conditions and sold from chill cabinets. Many of these products are acidified by the addition of mayonnaises or other vinegar-based dressings which contribute to the inhibition of microbial spoilage. Other products consist of prepared raw vegetables without any acidified dressing. These are commonly mixed, chopped, sliced or grated vegetables, sometimes with the addition of nuts and dried fruit. These latter products fall into the low acid range and are therefore, able to support the growth of a wide range of micro-organisms.

Vegetable salad ingredients (lettuce, tomatoes, broccoli, and cauliflower) purchased from three grocery-store deli operations were analyzed for total plate count, coliforms, yeasts, and molds. The total aerobic count for the vegetables ranged from 5.51 to 6.63 log CFU/g. Coliforms on the vegetables ranged from 4.89 to 6.30 log CFU/g. Yeasts and molds were found on all vegetables (Albrecht et al., 1995). Samples, comprising different types of raw vegetables (seven), fruits (three), and sprouts (three) obtained from street vendors were tested for aerobic plate count, coliform count and various food-borne pathogens. Average aerobic plate counts for salad vegetables, fruits, and sprouts were greater than 10^{10} and 10^9 cfu/g, respectively. *S. aureus*, *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *S. typhi*, *Serratia* spp., *Providencia* spp. and *P. aeruginosa* were isolated.

The antibiotic resistance patterns of the isolates revealed *P. aeruginosa* to be the most antibiotic resistant,

and *E. coli*, *Salmonella*, *Enterobacter aeruginosa* and *P. aeruginosa* also showed the presence of plasmids (Poorna and Randhir, 2001). Microbial quality of minimally processed leafy salads collected from retailers in the city of Sao Paulo, Brazil was evaluated for counts of total coliforms, fecal coliforms, *Enterobacteriaceae*, psychrotrophic microorganisms, and *Salmonella* for 133 samples. Populations of psychrotrophic microorganisms $>10^6$ CFU/g were found in 51% of the 133 samples, and *Enterobacteriaceae* populations between 10^5 and 10^6 cfu/g were found in 42% of the samples. Fecal coliform concentrations higher than 10^2 cfu/g (Brazilian standard) were found in 97 (73%) of the samples, and *Salmonella* was detected in 4 (3%) of the samples. The results indicated that minimally processed vegetables had poor microbiological quality, and these products could be a vehicle for pathogens such as *Salmonella* and *L. monocytogenes* (Fröder et al., 2007; Sher et al., 2010a).

Due to health concerns, food lifestyle has changed among Saudi population and special attention has been paid to eating salads. A recent nation-wide survey on the dietary habits of the Saudi population (Anonymous, 1993) revealed that eating green salad is a daily habit of about 50% of the individuals surveyed throughout the country. The only report available on vegetable salad ingredient from Saudi Arabia was on five salad vegetables, namely: parsley, lettuce, green onion, carrots and cucumber, collected from local markets of Riyadh city of Saudi Arabia, for the presence of total and faecal coliforms, salmonellae and *L. monocytogenes* (Ibrahim, 1996). The study reported that total coliform counts ranged from 1.4×10^4 cfu/g for cucumber to as high as 3.9×10^6 cfu/g for parsley. The mean faecal coliform counts ranged from 5.3×10^2 cfu/g to as high as 5.4×10^4 cfu/g for cucumber and parsley respectively. Salmonellae were detected in 6, 5, 4.8 and 4.5% of lettuce, cucumber, green onion and parsley samples, respectively. Presumptive *L. monocytogenes* was detected in parsley (44%), lettuce (12%) and green onion (8%).

Riyadh, the Saudi Arabian capital city, is fast developing with a population rapidly escalating. As a result the hotel and food industry is flourishing at faster rate in the city as well as in the Kingdom. The city houses more than 2170 restaurants, where several kinds of popular Arabian salads and others are served including: Tabbouleh, Fattoush, Hummus, Mutabbel and Caesar. Mutabbel known as eggplants salad is a popular salad in Turkey and Greece. Hummus is a popular dish for vegetarians and meat eaters alike. In Middle Eastern countries cuisine Hummus is considered as an appetizer. The Caesar salad is also popular in many countries around the world. The primary ingredients in these salads are vegetables, toasted or fried pieces of pita bread, and mashed chickpeas. Among these salads only Caesar salad is prepared fresh without any minimal processing, while the other salads are minimally processed before being served. Tabbouleh and Fattoush are popular

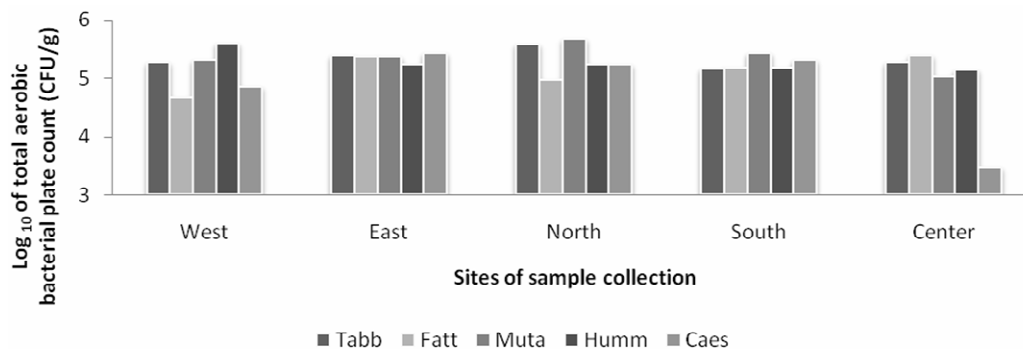


Figure 1. Total aerobic bacterial plate count of vegetable salads collected from restaurants, Tabb: Tabbouleh, Fatt: Fattoush, Humm: Hummus, Muta: Mutabbel, Caes: Caesar.

salads in many countries. They are popular in Brazil and in the Dominican Republic, due to Middle Eastern emigrants who settled there.

In the context of growing awareness on microbial quality of fresh and raw vegetables in salads the present study was conducted to investigate the bacteriological quality of such food items in Riyadh. The current study was particularly designed to include indicator organisms, food poisoning pathogens, in order to ascertain the food hygiene and public health. A comparative analysis was also made between salads prepared and served in restaurants versus similar home-made salads, and the results are presented in the present communication.

MATERIALS AND METHODS

Sample collection

Five kinds of salads which included Tabbouleh, Fattoush, Hummus, Mutabbel, and Caesar were subjected to microbiological analysis. Details of constituent ingredients that make the salads are shown in Table 1. Except Caesar which is also quite common in International Western restaurants as well as the Arab world, all other salads are popular dishes served in the Arab world. The samples were collected from various restaurants located in five distinct areas of the city including: west, north, south, east, and center of Riyadh city. A total of 200 salad samples were collected and analyzed. Forty samples were collected on a weekly basis during the months of August and November 2008. From each area eight samples of different salads were collected in sterile containers and analyzed within 1 h of collection. Apart from the samples collected from restaurants, homemade salads of the same types were also collected from selected homes and analyzed for a comparison.

Microbiological analysis

Samples were purchased in their original packages, put into plastic bags and transported to the laboratory. Samples were analyzed, as and when purchased. Duplicate samples were drawn and added to sterile peptone water or enrichment broth to make 10^{-1} dilution in a sterile 400 ml capacity stomacher bags. All samples were blended in Stomacher 400 (Tecmar Co. Cincinnati OH) for 2 min. and then 10 fold serial dilutions were prepared with buffered peptone water (BPW). Total count of all non fastidious and fastidious

microorganisms were estimated using Tryptic Soy Agar (TSA) (DIFCO), at 37°C/24 h. *E. coli* and total coliforms were enumerated by performing aerobic plate count using DIFCO-Violet Red Bile Agar containing lactose as fermentable sugar and incorporated with 4-methylumbelliferyl- β -D-glucuronide (MUG) (as recommended by DIFCO agar media for enumerating *E. coli* and total coliform bacteria in food and dairy products) at 37°C for 24 h. Presence of faecal coliform was determined using Brilliant Green Lactose Bile broth (DIFCO) at 44.5°C/ 48 h, followed by confirmation of gas positive tubes using Eosine Methylene Blue broth (DIFCO). On Violet Red Bile Agar with MUG media, coliform bacteria form purplish-red colonies that are generally surrounded by a reddish zone of precipitated bile. When colonies are examined under long-wave fluorescent light, colony surroundings showed a bluish fluorescent halo. *E. coli* colonies are red surrounded by a zone of precipitated bile and fluoresce blue under long-wave UV light. *E. aerogenes* are pink colonies surrounded by a zone of precipitated bile and fluoresce blue under long-wave UV light (Kornacki and Johnson, 2001; Hitchins et al., 1995; Feng and Hartman, 1982). For enumerating *Salmonella* and *Shigella* the remaining BPW was then incubated at 37°C for 24 h. SS Agar and *S. shigella* Agar are designated as moderately selective media based upon the degree of inhibition of Gram-positive microorganisms. *Salmonella* colonies were colorless with a black center, while *Shigella* colonies appear fully colorless.

Antibiotic sensitivity test

Antimicrobial susceptibility of the isolated *Salmonella* and *Shigella* was carried out by disc diffusion method on Mueller-Hinton agar using commercial antibiotics (μ g/disc) (Bauer, Kirby, Sherris and Turck, 1966). Antibiotics used, and their concentrations were as follows: ampicillin (10 μ g), amoxicillin (10 μ g), amikacin (30 μ g), cefotaxime (30 μ g), ceftizoxime (30 μ g), ceftriaxone (30 μ g), cephalothin (30 μ g), chloramphenicol (30 μ g), gentamicin (10 μ g), kanamycin (30 μ g), streptomycin (10 μ g), trimethoprim (5 μ g), and tetracycline (30 μ g). Results were recorded by measuring the inhibition zones and scored as susceptible or resistant according to the recommendations of the Clinical and Laboratory Standards. *E. coli* ATCC 25922 was used as a control organism.

RESULTS AND DISCUSSION

The data presented in Figure 1 for 200 samples of salads collected from restaurants demonstrated that the total aerobic bacterial counts, associated with the vegetable

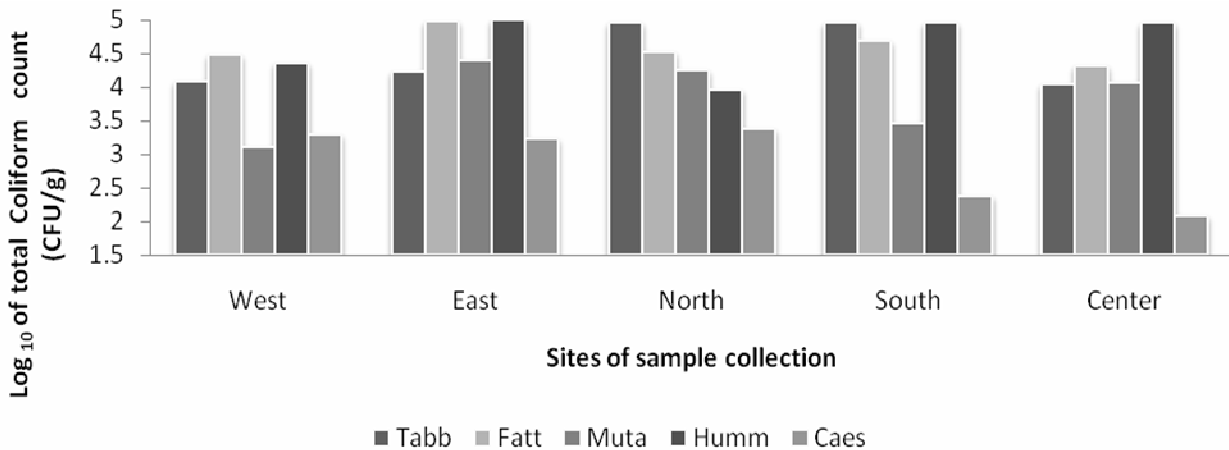


Figure 2. Total coliform bacterial count of vegetable salads collected from restaurants, Tabb: Tabbouleh, Fatt: Fattoush, Humm: Hummus, Muta: Mutabbel, Caes: Caesar.

salads, varied from 1.0×10^5 cfu/g to 4.5×10^5 CFU/g. The maximum microbial load was recorded for Mutabbel salads collected from the restaurants located in the northern part of Riyadh city. In general, most of the samples harbored bacterial load around 2×10^5 cfu/g. The total coliform counts in all five types of salads is presented in Figure 2. It was obvious that the samples from different restaurants in Riyadh were contaminated with coliforms (Figure 2). Coliforms are potential human pathogens and they were found in samples before the time they were served. Such a contamination might be from the origin of salad growing or might have occurred during processing of the vegetables and respective salad ingredients (Kornacki and Johnson, 2001). The colony counts for the total coliforms were in the range of $2-8 \times 10^4$ cfu/g. Higher level of cfu/g of total coliforms was recorded in Tabbouleh salads collected from the northern and southern parts, Fattoush from eastern part, and Hummus from eastern, southern and central parts of Riyadh city when compared to other locations. It was also observed that the salad "Caesar" served in the restaurants located in the western, southern and central areas of Riyadh city were not contaminated.

It is worth mentioning that irrespective of the kind and sampling location, all salads were contaminated with *E. coli* except Caesar salad. The level of contamination was dependent on the time period after sampling. Higher level of fecal contamination with *E. coli* was recorded in all Tabbouleh and Mutabbel salads collected from the restaurants located in the southern and eastern areas of Riyadh city. *E. aerogenes* was also found in all Tabbouleh and Mutabbel salads collected from the restaurants located in the southern and eastern areas of Riyadh city. However, it was not recorded in the samples of Hummus salad collected from the restaurants in the northern area, and in Tabbouleh and Fattoush salads collected from the restaurants located in Central part of

Riyadh. *Shigella* spp. and *Salmonella* spp. were not encountered in all types of salad samples analyzed during current study. However, *Shigella* contamination occurred in 25% Mutabbel and 12.5% of Hummus salads collected from the eastern part of Riyadh city. On the other side, *Salmonella* contamination was observed in Tabbouleh (37.5%), Fattoush (37.5%), and Mutabbel (25%) salads obtained from different restaurants in southern part of Riyadh city. Samples of Tabbouleh, Fattoush, and Hummus salads (12.5% each) collected from eastern part of Riyadh, in addition to Mutabbel salad, collected from the central part of Riyadh city restaurants were also found contaminated with *Salmonella*. Both the genera of *Salmonella* and *Shigella* were susceptible to most of the antibiotics tested for sensitivity except Trimthoprim and Tetracycline against which resistance was recorded. *Shigella* in addition, was resistant to Ampiciline as well (Bauer et al., 1966; Xanthopoulos et al., 2009).

All home-made salad samples were subjected to antimicrobial testing according to the defined parameters and the results are presented in Table 1. Total aerobic bacterial plate count for all 75 samples was found to be in the range of 5×10^4 to 12×10^4 cfu/g of salads. Mutabbel salad recorded the highest, while Fattoush showed the lowest total coliform counts. *E. coli* was recorded in 87.5% of the Mutabbel salads followed by Fattoush, and Hummus (67.5% each), and Tabbouleh (50%). Comparatively, only 12.5% of the Caesar salad recorded *E. coli*. Interestingly, all types of homemade salads recorded absence of *Shigella* spp. except one sample of Caesar salad was positive for *Shigella* sp.

Several investigations have been carried out on vegetable salads, their composition, medicinal importance, and were reported from different parts of the world (Down, 1995; Syyah et al., 2004; Al-Binali et al., 2006; Sher and Alyemeni, 2010). All those reports dealt

Table 1. Recovery of bacterial entropathogens from the different kinds of vegetable salads collected from restaurants in Riyadh city.

Kinds of salads and area of collection	Entropathogens			
	<i>E. coli</i> ^a (%)	<i>E. aerogenes</i> (%)	<i>Shigella</i> sp. (%)	<i>Salmonella</i> sp. (%)
West				
Tabbouleh	^b 3(37.5)	3(37.5)	-	-
Fattoush	4 (50)	4(50)	-	-
Mutabbel	3 (37.5)	3(37.5)	-	-
Hummus	3 (37.5)	3(37.5)	-	-
Caesar	-	-	-	-
East				
Tabbouleh	5 (62.5)	5(62.5)	-	3(37.5)
Fattoush	3(37.5)	3(37.5)	-	3(37.5)
Mutabbel	8(100)	8(100)	2(25)	2(25)
Hummus	4(50)	2(25)	1(12.5)	-
Caesar	1(12.5)	1(12.5)	-	-
North				
Tabbouleh	4(50)	3(37.5)	-	-
Fattoush	5(62.5)	4(50)	-	-
Mutabbel	3(37.5)	2(25)	-	-
Hummus	4(50)	-	-	-
Caesar	4(50)	2(25)	-	-
South				
Tabbouleh	8(100)	8(100)	1(12.5)	1(12.5)
Fattoush	3(37.5)	3(37.5)	-	1(12.5)
Mutabbel	6(75)	4(50)	1(12.5)	-
Hummus	4(50)	4(50)	1(12.5)	1(12.5)
Caesar	-	-	-	-
Center				
Tabbouleh	3(37.5)	-	-	-
Fattoush	2(25)	-	-	-
Mutabbel	5(62.5)	1(12.5)	-	1(12.5)
Hummus	2(25)	1(12.5)	-	-
Caesar	-	-	-	-

^aTypical colonies according to manufacturer. *E. coli* – Red, *E. aerogenes*- Pink, *Shigella* sp. - colourless, *Salmonella* sp. - colorless with black center, ^bNumber of positive sample out of eight samples.

with the individual vegetable components such as carrot, cucumber, parsley, lettuce, lemon etc. Whereas, the present study considered the prepared whole vegetable salads which are prepared after minimal processing (Table 2) and are quite popular in Arab world as popular dish being served in restaurants, hotels and made at homes. It may be noticed that the ingredients used in these salads are very different and only one or two vegetable ingredient are used in more than one kind of salad. Of the 5 kinds of the salads, the Caesar salad is normally prepared fresh by chopping the vegetables, mixed, and served. There is minimal processing of the

vegetable ingredients prior to mixing and serving. It is quite natural that the personnel involved in the preparation of these vegetables use their hands in the minimal processing and pretreatment of the ingredients before the final dish is made ready for servings. There is potential threat for safety as the microbial quality of these salads could be compromised while they are prepared and there could be possible contamination by fecal coliforms and other related human pathogens subject to the condition that the personal hygiene and sanitation in the kitchen and food processing are strictly regulated and controlled.

Table 2. Kinds of salad available in Riyadh city and the ingredients.

Kinds of salad	Ingredient of salads
Tabbouleh	Parsley, bulgur, mint, tomato, scallion, herbs, lemon, black pepper, cinnamon, allspice, lettuce
Fattoush	Radish, Tomato, Lettuce, Cucumber, Onion, Sumac, Mint, Olive oil, Lemon Juice, Salt, Pita - toasted or fried
Hummus	Chickpeas, tahini, olive oil, lemon juice, salt, garlic
Mutabbel	Eggplant, tahini, salt, pepper, olive oil, anar seeds
Caesar	Romaine Lettuce, croutons, parmesan cheese, lemon juice, olive oil, egg, Worcestershire sauce, black pepper

In this context current study was conducted in order to ascertain that these delicious vegetable salads are safe for human consumption and they are free from potential food poisoning and toxin producing pathogens. The results obtained in this study testify that minimally processed vegetable salads have contamination by potential food borne pathogens compared to the salad Caesar which is not processed prior to formulation. Interestingly this Caesar salad collected irrespective of the location in the city did not record *E. coli* or any other pathogen, there by evidencing possible source of contamination by human intervention. May be that is the reason that Caesar salad did not record high levels of contaminations in those localities. However, these salads collected from the restaurants located in northern and eastern areas did record bacterial contaminants suggesting contamination during the handling of the vegetables by personnel (Figures 3 and 4). In the present study it was further observed that the total coliform counts were comparatively on the higher side compared to the microbial load recorded for the individual vegetables in Riyadh earlier (Ibrahim, 1996). It must be understood that the earlier study was on the samples collected from vegetable retail market and did not include the restaurant samples. Thus the present study strongly suggested that cause of contamination by fecal coliform and associated pathogens might mainly be due to hand processing of vegetables during minimal processing. Another study carried out in India on a total of 120 samples, comprising different types of raw vegetables (seven), fruits (three) and sprouts (three) obtained from street vendors, for aerobic plate count, coliform count and various food-borne pathogens reported that on average aerobic plate counts for salad vegetables, fruits and sprouts were greater than 10^{10} cfu/g and 10^9 cfu/g respectively and it was demonstrated that *S. aureus*, *E. coli*, *Enterobacter* sp., *Klebsiella* sp., *S. typhi*, *Serratia* sp., *Providencia* sp. and *P. aeruginosa* were present in certain cases (Poorna and Kaur, 2001).

Results of the present study also indicated that minimally processed vegetable salads had poor microbial quality and holds potential health risk due to presence of higher load of total coliforms, besides *Shigella* and *Salmonella*. *Shigella* and *Salmonella* were also investigated in another study where the folklore drugs and medicinal plants used in food were tested and several plants were found to possess promising activity against different microorganisms under that study (Khatibi et al., 1989; Sher and Alyemini, 2010). The comparative study made with salads of restaurants and the home-made salads clearly evidenced that the personal hygiene of the personnel and microbial quality of the water used for preprocessing of vegetables in the restaurants were definitely on the lower side of restaurants as compared to home-made similar salads where adequate care is normally taken while preparing food. Another major factor that could have contributed to the higher fecal contamination on the vegetable salads is the probable source of the vegetable ingredients. There are many different kinds of vegetable and spice components included towards making a specific kind of salad. Each ingredient might have come from different geographical source and could have undergone differential pretreatment during their post harvesting process before they reach the restaurants. Good care is needed for pretreatment and minimal processing which could eliminate bioburden potential of health risk. This conclusion is based on the fact that as compared to other salads, Caesar salad had no fecal coliform counts in all the samples assessed irrespective of the location in the city. It suggested that the probable microbial contamination might occur due to location of vegetable growth which might be different in different areas of Riyadh city. The other major factor contributing is improper hygiene during minimal processing in the restaurants before formulation of the salad dishes. Based on the current and earlier studies, it is concluded that strict food safety and control measures have to be



Figure 3. Total aerobic bacterial plate count of home made vegetable salads, Tabb: Tabbouleh, Fatt: Fattoush, Humm: Hummus, Muta: Mutabbel, Caes: Caesar.



Figure 4. Total coliform bacterial count of home-made vegetable salads, Tabb: Tabbouleh, Fatt: Fattoush, Humm: Hummus, Muta: Mutabbel, Caes: Caesar.

enforced in food industry, particularly in hotels and restaurants where fresh vegetables and fruits are being served as delicacy and millions of people take food (Jones et al., 2008).

Conclusion

The study indicated that minimally processed vegetable salads had potential health risk due to presence of fecal coliforms and associated pathogens due to hand processing of vegetables during processing. Therefore, more concern needs to be taken during preparation. The workers should be educated about the path of contamination and observed them during preparing the salads.

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