Assessment of the microbiological safety of salad vegetables from different Restaurants in Ilam

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ABSTRACT

Vegetables, used in preparing salads, are most important part of the diet. These materials are often irrigated by untreated urban wastewater. Wastewater, contaminated with ova of parasites, bacteria and protozoa, are used as fertilizers and it can cause a variety of infectious diseases. The purpose of this study is detection of microbial contamination of salad used in Ilam's restaurants. In this study, 42 samples were collected from all restaurants placed in Ilam city and transferred to the laboratory. Brilliant Green Medium, Trypton water and Coax reagent used for detection of *Escherichia coli*. Water broth, Selenit systein, Tetrationat, Salmonella-shigella agar and Briliant green was used for identification of *salmonella*. For detection of *Enterococcus*, KF agar medium containing a diphenyl Tetrazolium chloride was used. Sabro dextrose agar medium (SDA) was used for detection of mold and yeast and wet mount and concentration methods used for parasitology investigations. The results of this study indicate that about 66.66% of samples were infected with *Enterococcus*, 69% had *E. coli* contamination and 83.33% of samples were contaminated with yeast. Samples were negative for presence of *Salmonella* and mold (mold not more than 10^3). Parasites contamination of samples was (4 cases) 9.5% for *Giardia lamblia*, (10 cases) 23.8% for *Taenia* eggs, 31% (13 cases) for *Hymenolepis nana* 16.6% (7 cases) for *Entamoeba coli*. The results of this study showed that salads are contaminated with infectious agent and the use of appropriate disinfectants and washing the vegetables used in salad preparation is essential for controlling infectious diseases.

Keywords: salad; microbial contamination; restaurants; Ilam

INTRODUCTION

The essential element of healthy diet is uncooked (raw) vegetables. Throughout the process of planting to consumption, vegetables can become contaminated with different pathogens such as enteric bacterial, viral and parasites. The extent of contamination depends on several factors that include, among others, use of untreated wastewater and water supplies contaminated with sewage for irrigation, post-harvest handling, and hygienic conditions of preparation in food service or home settings [1-3]. But they have a protective effect against diseases.
Salad vegetables are rich in vitamins and minerals and consumed without any heat treatment, sometimes without washing and peeling and therefore the possibility of food borne diseases is more [4, 5]. The oral route is the most common and the most important way of human infection to different pathogens [6]. In addition different egg parasites can attach to vegetables such as lettuce and cabbage and do not leave with simple disinfection and in some cases may cause serious illness [7].
Also various bacterial infections such as *Salmonella* and *Vibrio cholera* are the main cause of diarrhea especially in summer and detected in these vegetables [8]. In a study in Ghana, the salad vegetables were contaminated with, *Streptococcus faecalis, Salmonella Typhi, Shigella* and *Pseudomonas* [8]. On the other hands, protozoan parasites infection such as Amoeboid, giardia and cryptosporidium, are reported as main agents for parasitic diarrhea in

different studies [9]. For example in the study of salad vegetables in Saudi Arabia the prevalence of *Entamoeba coli*, *Giardia lamblia* and *Blastocystis hominis* were 35.5, 31.6 and 17.1 percent was reported [9]. The aim of this study was to clarify the types of intestinal parasites and microbial pathogen in vegetables salads available in the various restaurants of Ilam.

MATERIAL AND METHOD

In this descriptive and cross-sectional study data were collected based on preliminary studies from health care centers during 2012. Due to the limited number of restaurants (14 restaurants) samplings was performed 3 times and every 3 months and a total of 42 samples of fresh salad vegetables were collected during their preparation in restaurants. With regard to infectious agents, including bacteria, fungi, parasites and viruses, different methods were applied for detection of microorganisms.

Parasitological Examinations

Each fresh vegetable sample was weighted (100 g) into sterile plastic bags and washed with physiological saline solution (0.85% NaCl). The washing water was left for 10 h for sedimentation to take place and then the top layer was discarded and the remaining washing water centrifuged at 2164 g for 15 min. The supernatant was discarded, the residue carefully collected and examined in lugol stained slides through light microscopy. Cysts and eggs of parasites found under the microscope were identified.

Bacteriological Identification

A stock solution of 10-1 dilution was prepared by taking 20 g of each food sample and mixed with 180 ml of sterile phosphate-buffered saline and homogenised in a sterilized Warring blender for 5 min. The suspension was filtered through sterile filter paper (Whatman No. 2), and the filtrate received in a sterilized labeled conical flask, and used for subsequent tests.

Samples were examined by 25 Official Control Laboratories using Health Protection Agency (HPA) Standard Microbiological Methods [10-13]. The presence of *Salmonella* spp., *E. coli* and *Enterococcus* were enumerated for salad vegetable samples (table 1).

Mold & Yeast Identification

For the detection of mold and yeast Sabouraud's dextrose agar medium was used. For each sample, we prepared a plate containing medium, and added 1 cc of 0.001 dilution to the plate and it has spread very well and finally incubated at $25^{\circ C}$ for 24 -48 hours. After this period, the number of colonies that grew on the medium was counted.

Statistical Analysis

Descriptive and statistical analysis of the data, including the evaluation of risk factors, was undertaken using SPSS version 13.0. Relative proportions were compared using the chi-squared ($\chi 2$) and Fisher's exact tests. A probability value of less than 5% was deemed to be significant.

RESULTS

In this study we analyzed 42 samples of fresh salad vegetables were collected during their preparation in restaurants and found that due to high level of microbial agents samples are unsatisfactory. Of the 42 salad vegetables sampled, 98% (41) were of unsatisfactory microbiological quality due to *E. coli* ($\geq 10^2$ cfu g^{-1}, 69%), or the presence of *Enterococcus* (66.7%) but *Salmonella* spp. was not found in all samples studied. Yeast infection was reported in 33.83% of samples. There result indicated that *E.coli* was found to be predominant and observed in 29 samples of cabbage and carrot. The *Enterococcus* was statistically significant presented on lettuce and tomato respectively ($P<0.004$) (table 2).

Helminth eggs and *Giardia* cysts were detected in 55% (23/42) of fresh salad vegetables examined. These include 14% (5/23) of tomato, 96% (22/23) of lettuce and 100% of cabbage and carrot samples. Cabbage, carrot and lettuce samples were contaminated significantly more often than those of tomato samples with intestinal parasites ($P < 0.009$).

Table 1. Microbiological criteria for salad vegetables according to HPA guidelines

Micro-organism	Results (cfu g^{-1} unless otherwise specified)	Interpretation of microbiology quality
Escherichia coli	$\geq 10^2$	Unsatisfactory
Staphylococcus aureus	10^2 to $< 10^4$	Unsatisfactory
Salmonella spp.	Detected in 25 g	Unacceptable
Bacillus cereus	10^4 to $<10^5$	Unsatisfactory

Table 2. Microbiological quality of salad vegetables

Micro-organism		Number	percent	P Value
E.coli		29	69	NS
Enterococcus		28	66	NS
Salmonella		0	0	NS
Giardia		4	5.9	NS
Hymenolepis eggs		13	31	P<0.05
Taenia eggs		10	23.8	NS
Entamoeba coli		7	16.6	NS
Yeast	$<10^3$	7	16.6	NS
	$>10^3$	35	83.4	NS

NS: not significant

Table 3. Microbiology quality of salad vegetables

Mico-organism	salad vegetables type	Number (%)	P value
E.coli	Lettuce	2 (7)	P>0.05
	Cabbage	14(48)	
	Carrot	11(38)	
	Tomato	2(7)	
Enterococcus	Lettuce	15(53.5)	P= 0.004
	Cabbage	2(7.1)	
	Carrot	1(3.5)	
	Tomato	11(39.1)	
Giardia	Lettuce	2(50)	P>0.05
	Cabbage	1(25)	
	Carrot	1(25)	
	Tomato	0	
Hymenolepis eggs	Lettuce	5(38.4)	P= 0.001
	Cabbage	6(46.2)	
	Carrot	2(15.4)	
	Tomato	0	
Taenia eggs	Lettuce	4(40)	P>0.05
	Cabbage	5(50)	
	Carrot	1(10)	
	Tomato	0	
Yeast	Lettuce	7(50)	P>0.05
	Cabbage	3(21.4)	
	Carrot	3(21.4)	
	Tomato	1(7.2)	

Among 42 samples examined eggs of *Hymenolepis* eggs was detected in 13 samples (31%) and eggs of *Taenia/ Echinococcus* in 10 (23.8%) respectively. Cysts of *Giardia* spp. were detected in 9.5% (4 samples) and *Entamoeba coli* in 16.6% (7 samples) respectively. In addition, eggs of *Hymenolepis* eggs were detected significantly in total lettuce and cabbage samples than in tomato samples (P<0.001) (table 3).

DISCUSSION

Microbial contamination of salad vegetables can occur from the environment, from contact with contaminated containers, equipment and utensils, hands, cleaning cloths or pests [14-16]. Important microorganisms in food, including various species of bacteria, yeasts, molds and some parasites are capable of growth in food that can cause illness and food poisoning.

This study has shown that the majority of prepared salad vegetables in different restaurants in Ilam were of unsatisfactory/unacceptable microbiological quality according to published microbiological guidelines [17]. However, between 65-70% of salad vegetable sampled were shown to be of unsatisfactory or unacceptable microbiological quality due to high levels of *E.coli* and *Enterococcus.*

The incidence of *E. coli* in salad vegetables found in this study (69%) is twenty times higher than that found in an UK study of open prepared ready-to-eat salad vegetables from a range of different food service premises in 2001 (3%) [16]. Cenci-Goga et al. examined 894 samples for total counts of aerobic bacteria, counts of indicator organisms and pathogens and reported lower aerobic plate counts and a lower incidence of *S. aureus*, coliform organisms, *E. coli*, and *B. cereus*, whereas *Salmonella* spp. and *L. monocytogenes* were not detected in none of samples [18]. Similar to this study and study by Jalali et al. in Isfahan regarding *Salmonella* spp., we reported the same results [19]. In India out of 50 samples, 86 bacterial pathogens such as *E.coli* (38.3%), followed by *Enterobacter aerogenes* (20.9%), *Pseudimonas* (16.2%), *Staphylococcus aureus* (15.1%), *Salmonella* spp. (5.8%) and *Shigella* (3.4%) were isolated [4]. The authors suggested that strong washing of vegetables with sanitary running water is need before consuming them. As these studies mentioned, the main bacterial pathogen is *E.coli*, followed by *Staphylococcus aureus* and *Pseudimonas.*

On the other hands, intestinal parasites are very common in developing countries and fresh vegetables are an important direction of their transmission. In previous study by Daryani et al. in 2008, intestinal parasites were reported in 13 of 45 samples of native garden vegetables consumed in Ardabil [20]. In this study *Ascaris lumbricoids* eggs being detected in 2% of samples examined but we did not observe any positive samples in our study. We examined 42 samples of four different types of fresh salad vegetables from different restaurants in Ilam, of which 55% were positive for helminth eggs and *Giardia* spp. cysts. This result is compatible with Abougraina et al. study which presented 58% positivity for helminth eggs and *Giardia* spp. in Libya [21] but incompatible with study by Vosoughi and colleagues on vegetable intake in Shiraz's Restaurants [22].

Malakootian et al. have reported that the rate of parasitic contamination of vegetables in Kerman was 3.7% for *Hymenolepis* eggs, 2.9% *Dicrocoelium*, 2.22% *Taenia* eggs and in total 15.6% of the consumed vegetables [23]. These results indicate that vegetables are used for salads usually infected by *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and in most studies microbial contamination was belonged to spinach and radishes. In various studies, the main cause of agricultural contamination was wastewater. In this study, the main cause of contamination in Ilam restaurant salads is animal manure and irrigation of agricultural crops and vegetables with untreated sewage. Unfortunately, due to lack of adequate cleaning and disinfection of vegetables, contamination of vegetables salad was not diminished.

The only way to prevent the growth of spoilage and pathogenic microorganisms is follow the cold chain from production through distribution, but many of psychrophilic microorganisms can grow or survive at refrigerator temperature. Therefore these microorganisms can be the source of a Foodborne epidemic.

Thus the teaching of accurate detergent usage, personal hygiene, preservation of vegetables used in salads in cold conditions to prevent the growing of microorganisms that cause infection seems necessary.

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