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Microbiological analysis of ready-to-eat salads available at different outlets in Lahore, Pakistan

[1]Hannan, A., [1*]Rehman, R., [1]Saleem, S., [2]Khan, M. U., [1]Qamar, M. U. and [1]Azhar, H.

[1]*Department of Microbiology, University of Health Sciences Lahore, Pakistan*
[2]*Department of Biochemistry, University of Health Sciences Lahore, Pakistan*

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Abstract

Food-borne diseases are the global public health problem. These play a significant role in human morbidity, mortality and economic loss. Ready-to-eat salads are considered as a high-risk food because they do not require any heating, washing or cleaning prior to consumption. Therefore, we aimed to determine the microbiological quality of ready-to-eat salads in our locality. A total of 50 different salads were collected aseptically from different vendors and restaurants of Lahore, Pakistan. Each sample (10 g) was homogenized in stomacher. The homogenized material was serially diluted up to 10^{-6} using 0.1% peptone water as diluent. The dilutions were inoculated on blood, nutrient and MacConkey agar by Surface-Spread Plate technique and plates were incubated at 35°C for overnight. Aerobic colony count (ACC) was determined by counting the colonies on nutrient agar plates. The identification of the organisms was determined by their morphology, culture characteristics and biochemical profile. The ACC range of salad samples was found to be 1.0×10^3 cfu/g to 5.8×10^8 cfu/g. Among these, 22% samples showed unsatisfactory level of ACC and 20% were at borderline. The highest ACC (cfu/g) was found in dry vegetables salads (5.8×10^8) and least microbial loads (1.0×10^3) were observed in vinegar-containing vegetable salads. Among Gram-negative rods *Klebsiella* spp. (16%) were isolated most frequently followed by *Enterobacter* spp. (11%). Whereas among Gram-positive cocci, *Enterococcus* spp. (13%) was foremost followed by *Staphylococcus aureus* (7.5%). This study revealed the potential hazard of ready-to-eat salads and it is the need of the hour to perform a surveillance study at national scale.

Introduction

Foodborne diseases are a major global problem causing considerable morbidity and mortality annually (Hanson *et al*., 2012). World Health Organization (WHO) reported that every day more than 5000 children die globally due to consumption of contaminated food and water (Moeezuddin, 2005). Foodborne illnesses are prevalent in all parts of the world and their toll on human well-being is enormous which lead to major economic loss (Caroline *et al*., 2008). The incidence rate of foodborne diseases is also rising up. In industrialized countries about one-third of the population is suffering from foodborne illnesses each year whereas, in developing world the problem is worse due to overcrowding, poverty, inadequate sanitary conditions and poor general hygiene (Fratamico *et al*., 2005).

Ready-to-eat foods available in market have gained much popularity over the years because of the ease and the taste these offer. Despite of their advantages, the category ready-to-eat is considered as high-risk foods because they do not necessitate any heating or processing prior to consumption (Cruickshank, 1990). In Pakistan, different types of salads which are available ready-to-eat in a variety of vendors and restaurants, are admired consumables.

Fruits and vegetables are the main ingredients of these salads. These are exposed to microbial contamination through contact with soil, dust and water and by treatment at harvest or during postharvest handling. They therefore harbour various microorganisms including plant and human pathogens (Carmo *et al*., 2004). These raw vegetables and fruits, when used in salad preparation without sufficient washing, make salads unfit for human consumption. Moreover, use of treated wastewater for irrigation, lack of individual hygiene of food handlers, improper cleaning of storage and preparation areas and use of dirty utensils also contribute to contamination (Poorna and Randhir, 2001).

In previous studies conducted in different countries, pathogens isolated from several kinds of salads included *S. aureus*, *Escherichia 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 the

*Corresponding author.
Email: *raima.rehman01@gmail.com*
Tel: +923217877779

context of growing awareness on microbial quality of salads the present study was planned to investigate the microbiological quality of such food items in our setting.

Methodology

Prior to start of the study, the permission was taken from ethical review committee, University of Health Sciences, Lahore, Pakistan.

Sample size

At random 50 salad samples were collected from different vendors and restaurants of Lahore during the period of July to December 2012, and were transported to the Department of Microbiology, University of Health Sciences. The samples were transported and analyzed according to the FDA Bacteriological Analytical Manual (2003). The samples consisted of cream salads (n = 14), dry salads (n = 14), fruit salads (n = 7), vinegar-containing vegetable salads (n = 7), pasta salads (n = 6) and channa chat (n = 3). Samples were processed within 3 hours of purchase.

Samples processing

A 10 g of each sample was weighed and poured inside the stomacher bag along with 90 ml of 0.1% sterile peptone water. All the samples were blended in stomacher (400 Circulator, PBI International, Milan, Italy) at 300 rpm for 2 min.

Aerobic colony count (cfu/g)

The homogenized material was serially diluted from 10^{-2}, 10^{-3} to 10^{-6} using 0.1% sterie peptone water as a diluent. For each sample 100 µl of the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were inoculated by Surface-Spread Plate technique on Nutrient agar (in duplicate) for aerobic colony count (ACC) and on Blood and MacConkey agar for differentiation and isolation of bacteria. Using L-rod (hockey stick) the 100 µl drop was promptly spread on the surface of the agar.

The plates were allowed to dry for 15 min and were then incubated in inverted position for 24 hr. After the incubation period the plates were carefully examined. Duplicate nutrient agar plates of that dilution were selected that contained 30-300 colonies per plate. Colonies on both of the plates were counted and average of the two counts was taken to calculate aerobic colony counts. ACC were expressed as cfu/g.

Identification/purification of isolates

The identification of organisms was done on the basis of their cultural characteristics, colonial morphology, Gram staining and biochemical profile. The gram positive cocci were identified using catalase, coagulase and DNase tests whereas gram negative rods were confirmed by API-20E and API-20NE (BioMerieux, France).

Results

All the 50 samples were found to be contaminated with polymicrobial flora (Table 1). The ACC of all the samples ranged from 1.0×10^3 to 5.8×10^8 cfu/g (Figure 1). As per Public Health Laboratory Services (PHLS) guidelines, 22% of the samples showed unsatisfactory levels of ACC and 20% were at borderline while rest had satisfactory levels. Of the seven different types of salad samples, dry vegetable salads had the highest microbial load (5.8×10^8 cfu/g) while vinegar-containing vegetable salads had the least microbial load (1.0×10^3 cfu/g) (Table 1).

A total of 133 organisms were isolated from all salad samples (n = 50). Among Gram-negative rods (GNR) the predominant was *Klebsiella* spp. (16%), followed by *Enterobacter* spp. (11%), whereas among Gram-positive cocci the major isolate was *Enterococcus* spp. (13%), followed by *S. aureus* (7.5%) (Table 2).

Discussion

Foodborne illnesses are a growing public health problem worldwide and need serious attention being a major cause of personal distress, social disturbance, preventable death and avoidable economic burden (Scharff, 2012). In present study ACC (cfu/g) of salad samples ranged from 1.0×10^3 to 5.8×10^8 (Table 1) and 22% of salads showed unsatisfactory levels. According to our knowledge, there is no such data available on food salads in our setup so far. Whereas, studies from Togo (Adjrah *et al.*, 2013), Iran (Najafi and Bahreini, 2012) and Hong Kong (HKSAR, 2002) reported almost similar data which coincide with our study. In contrast, other studies from Saudi Arabia (Khiyami *et al*., 2011) and Nigeria (Osamwonyi *et al*., 2013) reported lower ACC (cfu/g). This difference could be due to the mishandling of food and disregard to hygienic measures by food vendors in our locality which may introduce contaminants and pathogens to food throughout the chain of food production, processing, storage and preparation.

In this study out of seven different types of salads, dry vegetable salads yielded maximum bacterial counts (5.8×10^8 cfu/g) while vinegar-containing salads showed minimal counts (1.0×10^3 cfu/g) (Table

Table 1. ACC and microorganisms isolated from different salad types

Type of salad (no. of samples)	ACC (cfu/g) minimum	maximum	Organisms isolated
Cream salads (n=14)	$2.0x10^4$	$1.0x10^8$	*Klebsiella spp., Micrococcus spp., Enterococcus spp., Enterobacter spp., Acinetobacter spp., Escherichia coli, Staph aureus, Citrobacter spp., Providencia spp., Pseudomonas aeruginosa* and *Pantoea spp.*
Dry vegetable salads (n=14)	$2.9x10^4$	$5.8x10^8$	*Klebsiella spp., Micrococcus spp., Enterococcus spp., Enterobacter spp., Acinetobacter spp., Escherichia coli, Staph aureus, Citrobacter spp., Bacillus spp., Diphtheroids, Providencia spp., Pseudomonas aeruginosa, Pantoea spp.* and *CoNS*
Fruit salad (n=7)	$3.0x10^5$	$4.1x10^8$	*Klebsiella spp., Enterococcus spp., Enterobacter spp., Acinetobacter spp., Escherichia coli, Staph aureus Citrobacter spp., Bacillus spp., Diphtheroids* and *Providencia spp.*
Channa chat (n=3)	$1.5x10^5$	$6.7x10^7$	*Klebsiella spp., Micrococcus spp., Enterococcus spp., Enterobacter spp., Citrobacter spp.* and *Bacillus spp.*
Salad in vinegar (n=7)	$1.0x10^3$	$1.6x10^7$	*Klebsiella spp., Micrococcus spp., Enterococcus spp., Enterobacter spp., Acinetobacter* spp., *Bacillus spp., Diphtheroids* and *Pantoea spp.*
Pasta salad (n=6)	$2.0x10^4$	$1.5x10^7$	*Klebsiella spp., Micrococcus spp., Enterococcus spp., Enterobacter spp., Acinetobacter spp., Staph aureus, Citrobacter spp., Bacillus spp.* and *Pseudomonas aeruginosa*

Table 2. Frequency of organisms in salad samples

Organisms (n=133)	n	%
Gram-negative rods (n=77)		
Klebsiella spp.	21	16
Enterobacter spp.	15	11
Acinetobacter spp.	12	9
Citrobacter spp.	10	7.5
E. coli	10	7.5
P. aeruginosa	3	2
Providencia spp.	3	2
Pantoea spp.	3	2
Gram positive cocci (n=46)		
Staph aureus	10	7.5
Micrococcus spp.	18	13.5
Enterococcus spp.	17	13
CoNS	1	<1
Gram positive rods (n=10)		
Bacillus spp.	6	4.5
Diphtheroids	4	3

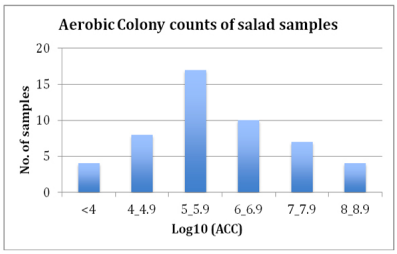

Figure 1. Aerobic Colony counts (ACC) of salad samples
The ACC are converted to base 10 logarithms.

2). These findings correlate with the two previous data published in Nigeria (Eni *et al*., 2010; Oranusi *et al*., 2013). High microbial loads might be due to the fact that vegetables are widely exposed to microbial contamination through contact with soil, dust, water and some farmers use animal manure or fecal as a fertilizer to enrich soil (Geldreich *et al*., 1962). On the other side, low count may perhaps due to the vinegar, which is a well renowned decontaminant (Eni *et al*., 2010).

In present study, *Klebsiella* spp., were the predominant organisms among GNR and *Enterococcus* spp. were the commonest organisms among GPC (table 2). These findings are almost in accordance with the previous studies conducted in Ota, Nigeria (Eni *et al*., 2010); Edo State, Nigeria (Osamwonyi *et al*., 2013) and USA (Macovei and Zurek, 2007). These organisms in salads indicate possible fecal contamination of food and poor hygienic processing practices (Tambekar *et al*., 2007). Handling of food with contaminated hands and utensils, insufficient washing of fruits and 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). According to our knowledge, there is no such data available on food salads in Pakistan so far.

Conclusion

This study revealed the potential hazard of ready-to-eat salads and emphasizes the need of surveillance studies at national scale. The present study supports that the quality of ready-to-eat salads available to public is not good as it is mandatory that foods must be free from contaminations as much as possible. Although these microorganisms can be part of the epiphytic flora of the fruits and vegetables used in salads, their persistence and proliferation is a reflection of poor hygienic practices by the food handlers.

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