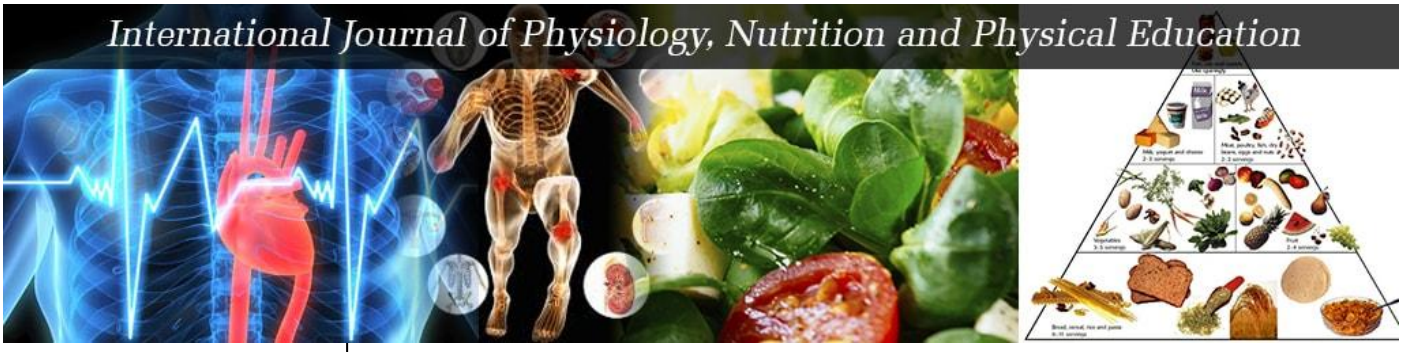

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Examination of microbiological quality of green salad served at selected restaurants

Prabhjot Kaur, Dr. Vinti Davar and Dr. Neelam

Abstract
Introduction: Food quality does not encompass only the sensory characteristics and presentation of food rather it is a comprehensive term in the sense that the food served to the guests must be nutritive as well as microbiologically safe so as to do no harm after consumption. But the ground realities are just the reverse of it.

Objectives

- To analyse microbiological adequacy of green salad served at the selected restaurants
- To identify pathogens present in green salad served thereto

Methodology: Microbiological quality examination of food samples collected from the selected restaurants was done using spread plate method, counting CFUs, physical examination of colonies, smear preparation, gram staining and microscopic examination of slides. Standardized recipes prepared were also analyzed for comparative analysis.

Results: Green salad samples from all three types of restaurants had shown high incidence of total viable counts. The highest bacterial CFU/ml in samples of green salad was however recorded as 7.6×10^{10} at 10^{-8} dilution in private restaurants followed by a mean CFU/ml value of 5.7×10^{10} and 4.0×10^{10} respectively in case of public and fast food restaurants at the same dilution. The pathogens found in samples of green salad from private restaurants include *E. coli, Staphylococcus aureus, Aspergillus, Penicillium* while those from public establishments had *Bacillus subtilis, Bacillus cereus, Aspergillus, Yeast*. However, *Bacillus subtilis, Staphylococcus aureus, Penicillium* were detected in case of fast food restaurants.

Conclusion: The p-values between private versus public, private versus fast food and public versus fast food restaurants did not favour significance in microbial colonies. Hence, it could be concluded that all three types of restaurants serve green salad with similar microbiological quality. This perhaps may be due the reason that salad being highly perishable is very difficult to be protected from microbial contamination. Thus, green salad has been reported responsible in maximum of the eating out food poisoning outbreaks.

Keywords: Microbiological examination, quality, pathogens, food poisoning, restaurants

Introduction

Food safety is a scientific discipline describing the handling, preparation, and storage of food to prevent food borne illness. Food safety is in one's hand. It can be ensured maintaining proper hygiene standards. So, both personal as well as work area hygiene is of utmost importance. Moreover, potential food safety hazards can be biological, chemical and physical. The safety of food is of utmost significance and has gained a worldwide attention. The globalization of food supply is major trend contributing to food safety problems. Tourism and increased cultural interest may lead to new eating habits. Eating away from home is a major trend in latest years. Many of the meals taken away from home require extensive food handling and /or are cold foods that are not cooked properly before consumption. Food safety and quality and consumer protection against the food fraud relate to basic human rights as advocated by FAO. Catering and food service play an increasingly important part in our experience of food quality and safety. Food control can be defined as the mandatory regulatory activity of the enforcement of food laws and regulations by national or local authorities to provide consumer protection and ensure all food during production, handling, storage, processing and distribution are safe, wholesome and fit for human consumption & conform to

safety and quality requirement and honestly labeled as prescribed by the law. The food control system is the official institutional set up, at national or sub national levels responsible for ensuring the safety and quality of the food supply. It includes: Food control management, Food laws, regulation and standards, inspection services, Good practices and Quality assurance, Laboratory services, Information, Education, Communication, and Training. Adulteration of foods also poses a serious danger to food quality and safety. In recent years, total quality management (TQM), Hazard Analysis and Critical Control Point (HACCP) and International Organization for Standardization (ISO) certification have assumed significant role.

There are very few pleasures in life and food is one of them. The trend of eating out shows a close correlation with lifestyle, social contacts and work patterns. The potential reasons people choose to eat out of the home include increased disposable income; celebrations; inability / unwillingness to cook; meetings / conferences; trying new tastes; emergency; traveling; entertaining and socialising. But eating out often means eating foods that are fat and calorie bombs due to large portion sizes and unhealthy cooking methods. Restaurant foods contain lots of calories, sugar, sodium and unhealthy fats hence they increase the risk of obesity, type 2 diabetes, high blood pressure and heart disease. Increased health risks are directly associated with increased consumption of restaurant foods. Food poisoning is commonly experienced in those who eat out frequently. Restaurants in general and chain restaurants in particular, often add many food chemicals to their meals. Special sauces and flavorings often contain sweeteners, flavor enhancers and hundreds of other additives. Eating out can cause illness in many ways. In many restaurants, food sits for several days in large refrigerators or worse, at room temperature for hours before being served. These items often harbor bacteria and other toxins as well as nutrients are lost. Food is often less fresh in restaurants because they buy more than is needed to avoid running out if they have a busy night. This means much is leftover, which increases the risk of spoilage and nutrient loss. Many restaurant workers are low-skilled employees who are in varying states of health. Most need their jobs and do not stay home if they are feeling ill. They may inadvertently sneeze, wipe their hands on their sleeve or take other actions that contaminate food, in spite of the apparent cleanliness of the establishment.

Review of literature

Food safety and food-borne infections are important public health concern worldwide and most of the pathogens resulting in food-borne diseases are zoonotic (Busani *et al*., 2006) [4]. *Staphylococcus aureus* is one of the most common agents in bacterial food poisoning outbreaks (Adwan *et al*., 2005) [2] and symptoms of staphylococcal food intoxication generally occur one to six hours after the food is ingested and the common symptoms are nausea, vomiting, abdominal cramps and diarrhea. Poultry, meat and egg products could be the common sources of S. aureus, posing a potential health risk. In developing countries, incidence rate of food borne diseases is approximately 916 cases per 100000 populations. Considering reports of W.H.O., economic loss posed by salmonellosis could be estimated about one billion dollar with medical and productivity costs taken into account (Pereira *et al*., 2009; Scallan *et al*., 2011) [12, 13]. Total aerobic bacteria, enterobacteriaceae, coliforms, and *Escherichia coli* are used as indicators of poor microbiological quality of food particularly face contamination (Abu-Ruwaida *et al*., 1994; Capita *et al*., 2002) [1, 5]. Thus, assessment of the chemical quality of these food products is very important to improve health of consumers (Jay, 2006) [9]. Therefore, it is important to prevent the hazards and to provide a safe and wholesome product for human consumption. Large number of catering services and restaurants seem necessary to be examined for hygienic quality (microbial contamination and chemical properties) of food stuff in these locations.

The concerns with restaurant food consumption in developing countries also include poor hygiene during preparation, storage and handling leading to microbiological contamination. Five star restaurant foods are also not always safe for consumption compared to homemade and restaurant foods, reported by Kampen in 1998 in Jakarta. In 2014, Nazni P. and Jaganathan A. have reported that multiple food items from street of Salem district of Tamil Nadu, India showed more viable microbial count (spores, yeast, Gram – ve rod and Gram + ve cocci) than same homemade food items, due to unhygienic food preparation and storage at inappropriate temperatures, exposure to flies, dust, wind and other contaminants.

A majority of food poisoning outbreaks is associated with improper holding that occurs in institutional settings (CDC 1996, 2000). Approximately 250 outbreaks involving 15,000 cases were reported to the Centers for Disease Control and Prevention from 1990-2003. The most effective system to control food safety within a processing plant is hazard analysis critical control point (HACCP), which is reliant on other programs including Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) and Pre-requisite Programmes (PRPs). Microbial analysis of environmental sampling of food production is more and more frequent. It is now clearly recognized that environmental control of food production plants is an important part of HACCP principles to prevent food contamination.

In 2008, a microbiological survey of ready-to-eat (RTE) filled baguettes, salads, cutting boards, selected utensils (preparation knives and serving spoons) and hands of food handlers in 4 retail delicatessens in Johannesburg, South Africa was conducted by Christison *et al*. All samples were analyzed using standard plating techniques. Similar counts of aerobic bacteria (9 log cfu/g), and coliforms and *Escherichia coli* (5-6 log cfu/g) were determined for filled baguettes and salads. *Staphylococcus aureus* (2 log cfu/g), *Bacillus cereus* (2 log cfu/g), *Salmonella* spp. (16 percent) and *Listeria monocytogenes* (4 percent) were also present in some of the RTE foods. Highest counts of aerobic bacteria were found on serving spoons (5.1 log cfu/cm) while highest coliforms and *Escherichia coli* were found on cutting boards (4 and 1.5 log cfu/cm, respectively). Microbial growth in utensils was above 100 cfu as per him. Knives' microbiological examination revealed presence of numerous bacteria ($8.6x10^5$cfu/knife) such as coliforms, *Staphylococcus aureus*, *Salmonella* and *Shigella*. During investigations on street food vendors' material, seventy samples of three types of dish washing water (E1, E2 and E3), eighty-five pieces of money, eighty utensils were collected for microbiological assessment. Hands' microbiological status of one hundred twenty-five consumers and seventy sellers were also assessed. The analysis revealed that 100 percent of E1 washing waters were very impure, while, 44.5 percent of second washing waters (E2) were impure, 44.5 percent very impure and 11 percent acceptable. 45.45 percent of E3 washing waters were acceptable, 27.27 percent impure and 27.27 percent very

impure. The spoons and the dinner plates were sometimes contaminated with unacceptable levels (above 102) of different bacteria such as coliforms and *Staphylococcus aureus*. Knives' microbiological examination revealed presence of numerous bacteria (8.6 x 10^5 cfus / knife) such as coliforms, *Staphylococcus aureus*, *Salmonella* and *Shigella*. Pieces of money analysis revealed presence of coli forms and *Staphylococcus aureus*. This data showed pathogen bacteria in food vending sites indicating hygiene monitoring failure (Barro *et al*., 2006) [3].

Materials and methodology

Apparatus

Autoclave	Laminar air flow
Micropipettor	Micropipettor tips of varying sizes
Sterile test tubes	Sterile petridishes
Conical flasks	Cotton swabs
Lab thermometer	Glass stirrer
Hot water bath	Flame burner
Colony counter	

Materials required

Peptone	Dextrose
Beef Extract	Potato Starch
Agar	Yeast Extract
NaCl	Chloramphenicol
Distilled water	Ethanol
Phenol	Lactic Acid
Cotton Blue	Crystal Violet
Gram's Iodine	Safranin

Preparation

Autoclaved water blanks
Nutrient Media
Autoclaved Agar plates

Sample Collection

Permission was sought from the restaurants and only 32 restaurants showed willingness to participate. Out of these, only 6 restaurants i.e. two private restaurants (R1), two public restaurants (R2) and two fast food restaurants (R3) were selected for microbiological analysis owing to the feasibility of sample collection. The food samples were procured from private, public and fast food restaurants in a sterile ice box. The standardized recipe was also formulated in consultation with chefs of different restaurants and prepared by the researcher in hygienic settings.

Photo 1: Isolation of Microbes after Incubation from Agar Plates

Photo 2: Studying the Colony Morphology of Bacterial Agar Plates

Method

Serial dilutions of food samples were prepared in already autoclaved water blanks. Inoculation of autoclaved agar plates was carried out by spread plate method. Microbiological quality examination of green salad samples collected from private, public and fast food restaurants was done by counting CFUs, physical examination of colonies (Photo 2), preparation of smears, gram staining and microscopic examination of the slides. The standardized recipe was also analysed using the standard procedure for comparative analysis.

The inoculation of collected samples was done in triplicates on Nutrient Agar (NA) for bacterial colonies and Chloramphenicol Yeast Glucose Agar (CYGA) for fungal colonies at specified serial dilutions (10^{-6} to 10^{-8} for bacterial growth and 10^{-2} to 10^{-5} for fungal growth) under sterile conditions in laminar air flow. This was thereafter followed by a controlled incubation at 37^0 C for a period of 24 to 48 hours for bacterial counts and for a period of 4 to 5 days for fungal counts on agar plates. The mean of bacterial and fungal CFUs was then calculated for all dilutions using SPSS version 16.0 The CFUs/ml were also calculated using the standard microbiological formula.

$$\text{CFU/ml} = \frac{\text{Number of CFUs} \times \text{Dilution Factor}}{\text{Volume of the sample inoculated}}$$

The methodology for preliminary microbiological analysis of food samples is summarized in the form of a flow chart (Fig.1).

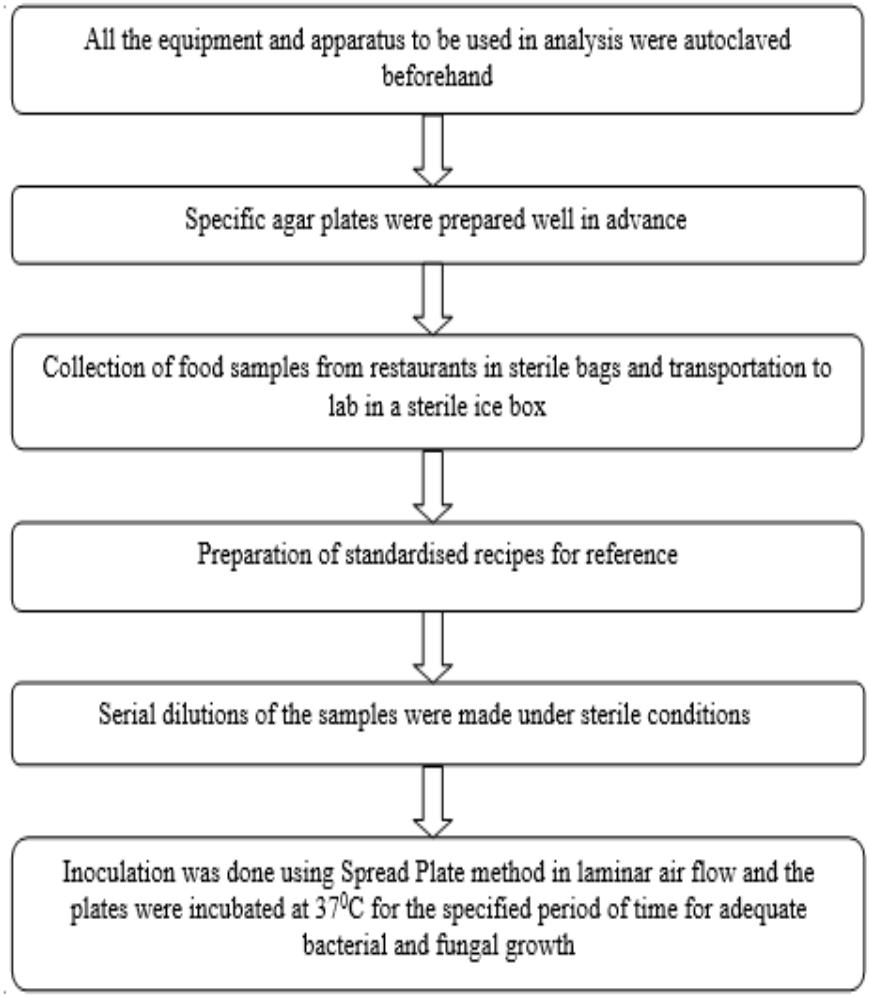

Fig 1: Methodology for Microbiological Analysis of Green Salad at a Glance

The standardized recipe prepared was also analyzed using the standard procedure for comparative analysis. The inoculation of collected samples was done in triplicates on Nutrient Agar (NA) for bacterial colonies and on Choloramphenicol Yeast Glucose Agar (CYGA) for fungal colonies at specified serial dilutions (10^{-6} to 10^{-8} for bacterial growth and 10^{-2} to 10^{-5} for fungal growth) under sterile conditions in laminar air flow. This was thereafter followed by a controlled incubation at 37^0C for a period of 24 to 48 hours for bacterial counts and for a period of 4 to 5 days for fungal counts on agar plates. The mean of bacterial and fungal CFUs was then calculated for all dilutions using SPSS version 16.0

Results and discussion

Collection of the samples of green salad from private, public and fast food restaurants was followed by microbiological analysis for bacterial as well as fungal contamination using standard procedures. The specified serial dilutions were then inoculated on agar plates in laminar air flow and incubated for a period of 24 to 48 hours for bacterial growth and for 4 to 5 days for fungal growth. The CFUs thus obtained were counted and fed to SPSS for calculation of mean values.

Microbiological Examination of Green Salad

Microbiological analysis was performed on the samples of green salad collected from the selected private, public and fast food restaurants. The table 1 illustrates the mean of bacterial and fungal CFUs in the studied samples procured from private, public and fast food restaurants.

Table 1: Comparison of Microbial Flora (CFU/ml) of Green Salad (GS) served at Private, Public and Fast Food Restaurants

GS	Dilution	Mean Bacterial CFU**	Bacterial CFU**/ml	Dilution	Mean Fungal CFU**	Fungal CFU**/ml
R1	10^{-6}	299	2.99×10^9	10^{-4}	1	1×10^5
	10^{-7}	190	1.90×10^{10}	10^{-5}	0	0
	10^{-8}	76	7.6×10^{10}	10^{-6}	1	1×10^7
R2	10^{-6}	215	2.15×10^9	10^{-4}	3	3×10^5
	10^{-7}	145	1.45×10^{10}	10^{-5}	2	2×10^6
	10^{-8}	57	5.7×10^{10}	10^{-6}	2	2×10^7
R3	10^{-6}	187	1.87×10^9	10^{-4}	3	3×10^5
	10^{-7}	98	9.8×10^9	10^{-5}	2	2×10^6
	10^{-8}	40	4.0×10^{10}	10^{-6}	2	2×10^7
SR*	10^{-6}	22	2.2×10^8	10^{-4}	1	1×10^5
	10^{-7}	13	1.3×10^9	10^{-5}	1	1×10^6
	10^{-8}	9	9×10^9	10^{-6}	1	1×10^7

*Standardised Recipe, ** Colony Forming Units

The samples of green salad obtained from the selected private restaurants have mean scores for bacterial colonies as 299, 190 and 76 respectively at 10^{-6}, 10^{-7} and 10^{-8} dilution. The mean values for bacterial colonies in samples of public restaurants are 215, 145 and 57 at the same dilutions. 187, 98 and 40 bacterial colonies have been observed by the researcher in green salad samples collected from fast food restaurants. However, the mean scores for the standardized recipe marked 22, 13 and 9 colonies only. On analyzing the fungal colonies, it was found that both public and fast food restaurants have same values for mean fungal colonies at the specified dilutions. These values have been observed lower in the samples of private restaurants with mean fungal colonies of 1, 0 and 1 respectively at 10^{-4}, 10^{-5} and 10^{-6} dilution. Interestingly, the standardized recipe has a mean of 1 at all the specified dilutions. The researcher observed that green salad served at the private restaurants (R1) had the highest number of bacterial colonies on their plates after incubation period.

The highest bacterial CFU/ml was recorded as 7.6×10^{10} at 10^{-8} dilution in private restaurants followed by a mean CFU/ml value of 5.7×10^{10} and 4.0×10^{10} respectively in case of public and fast food restaurants at the same dilution. In comparison to fast food restaurants (R3), 1.90×10^{10} and 1.45×10^{10} bacterial CFUs per ml. respectively were recorded at 10^{-7} dilution in green salad served at private (R1) and public (R2) restaurants. However, lesser growth was noted at 10^{-8} dilution with the standardized recipe of green salad. The heavy bacterial growth on all the samples of green salad may easily be associated with the high incidence of food poisoning cases resulting from consumption of green salad at such eateries. While evaluating the mean fungal CFUs of green salad, almost similar results were observed at private restaurants (R1) and standardized recipe. Even public restaurants (R2) showed same fungal CFUs as fast food restaurants (R3) at all the dilutions.

Table 2: Comparison of Bacterial CFUs in Green Salad served at Private, Public and Fast Food Restaurants

T-Test		Type of Restaurants	T	DF	Sig. (2-tailed)
CFU	Equal variances assumed	Private versus Public	.298	16	.770
	Equal variances not assumed		.298	13.947	.770
CFU	Equal variances assumed	Private versus Fast Food	1.283	16	.218
	Equal variances not assumed		1.283	15.906	.218
CFU	Equal variances assumed	Public versus Fast Food	.733	16	.474
	Equal variances not assumed		.733	14.546	.475

While appraising the results of t-test performed on the various samples of green salad collected from private, public and fast food restaurants, it was noted that none of the comparisons have shown any significant difference in the microbiological quality of the green salad (Table 2). The p-values of 0.770, 0.218 and 0.474 respectively at 95 percent confidence level between bacterial colonies of private versus public, private versus fast food and public versus fast food restaurants do not favour significance. Hence, it can be concluded that all the three types of restaurants serve green salad with similar microbiological quality. This perhaps may be due the reason that salad being highly perishable is very difficult to be protected from microbial contamination.

It was evident from the table 3 that there exists a highly significant difference in fungal CFUs per ml. in the samples of green salad taken from private, public and fast food restaurants. The p-value of 0.000 at 99 percent confidence level marked a highly significant difference in both private versus public restaurants and private versus fast food restaurants of this study. But not any significance has been observed at public versus private restaurants according to the outcomes of t-test with a p-value of 1.000 at the same level of confidence. This reveals that same favourable conditions for the growth of fungus were present in restaurants of both of these types.

Table 3: Comparison of Fungal CFUs in Green Salad served at Private, Public and Fast Food Restaurants

t-test		Type of Restaurants	t	df	Sig. (2-tailed)
CFU	Equal variances assumed	Private versus Public	-7.071	16	.000**
	Equal variances not assumed		-7.071	16.000	.000**
CFU	Equal variances assumed	Private versus Fast Food	-7.071	16	.000**
	Equal variances not assumed		-7.071	16.000	.000**
CFU	Equal variances assumed	Public versus Fast Food	.000	16	1.000
	Equal variances not assumed		.000	16.000	1.000

**Significant at $p \leq 0.001$

Plate 1: Bacterial Colonies on NA Plate of Green Salad served at Private Restaurants (10^{-6} Dilution)

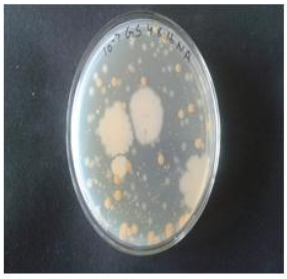

Plate 2: Bacterial Colonies on NA Plate of Green Salad served at Private Restaurants (10^{-7} Dilution)

Plate 3: Bacterial Colonies on NA Plate of Green Salad served at Private Restaurants (10^{-8} Dilution)

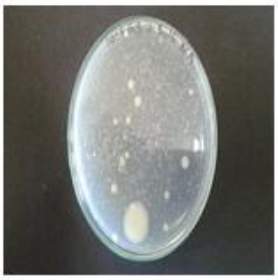

Plate 4: Bacterial Colonies on NA Plate of Green Salad served at Public Restaurants (10^{-6} Dilution)

Plate 5: Bacterial Colonies on NA Plate of Green Salad served at Public Restaurants (10^{-7} Dilution)

Plate 6: Bacterial Colonies on NA Plate of Green Salad served at Public Restaurants (10^{-8} Dilution)

Plate 7: Bacterial Colonies on NA Plate of Green Salad served at Fast Food Restaurants (10^{-6} Dilution)

Plate 8: Bacterial Colonies on NA Plate of Green Salad served at Fast Food Restaurants (10^{-7} Dilution)

Plate 9: Bacterial Colonies on NA Plate of Green Salad served at Fast Food Restaurants (10^{-8} Dilution)

Plates 1 to 9: are the results of the bacterial colonies found on the agar plates of green salad obtained from private, public and fast food restaurants after incubation period of 24 -48 hours at 37^0 C.

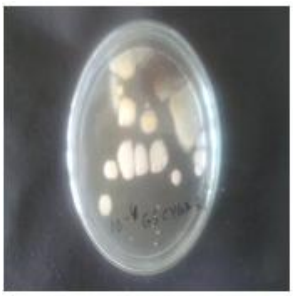

Plate 10: Fungal Colonies on CYGA Plate of Green Salad served at Private Restaurants (10^{-4} Dilution)

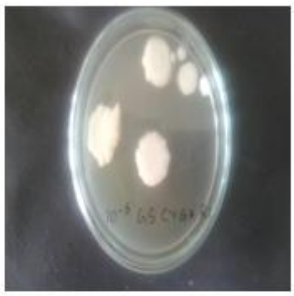

Plate 11: Fungal Colonies on CYGA Plate of Green Salad served at Private Restaurants (10^{-5} Dilution)

Plate 12: Fungal Colonies on CYGA Plate of Green Salad served at Private Restaurants (10^{-6} Dilution)

Plate 13: Fungal Colonies on CYGA Plate of Green Salad served at Public Restaurants (10^{-4} Dilution)

Plate 14: Fungal Colonies on CYGA Plate of Green Salad served at Public Restaurants (10^{-5} Dilution)

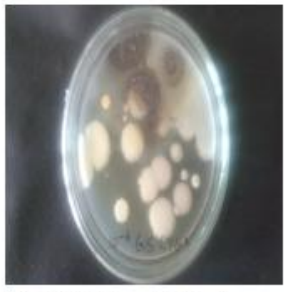

Plate 15: Fungal Colonies on CYGA Plate of Green Salad served at Public Restaurants (10^{-6} Dilution)

Plate 16: Fungal Colonies on CYGA Plate of Green Salad served at Fast Food Restaurants (10^{-4} Dilution)

Plate 17: Fungal Colonies on CYGA Plate of Green Salad served at Fast Food Restaurants (10^{-5} Dilution)

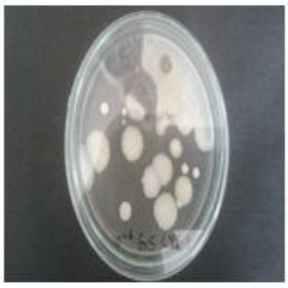

Plate 18: Fungal Colonies on CYGA Plate of Green Salad served at Fast Food Restaurants (10^{-6} Dilution)

Plates 10 to 18: represent the agar plates of fungal colonies on various samples of green salad procured from the selected restaurants. These CFUs were counted from these plates and the fungi were then isolated on fresh agar plates for further examination.

Morphological examination of the microbial colonies

Tables 4 depicts the results of morphological features examined by the researcher to help in the identification of the microbes found on various plates of the studied samples of selected recipes from the private, public and fast food restaurants.

Preparation of smears

a) Preparation of Bacterial Smears

The isolated bacterial colonies obtained on agar plates were then transferred to nutrient broth tubes following standard procedures under sterile conditions. The cultures were then incubated at 37^0C for 24 to 48 hours. The pure bacterial cultures thus obtained were mounted on sterilized slides by the standard smear preparation procedure.

Table 4: Morphological Examination of the Bacterial Colonies in Green Salad served at Private Restaurants

Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
GS	10^{-6}	1	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright/ Yellow	Opaque
		2	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
		3	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
	10^{-7}	1	Circular Irregular	Entire Undolate	Raised Flat	Punctiform/Medium Large	Smooth Smooth	Shiny Cloudy	White/Yellow White	Opaque Translucent
		2	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
		3	Circular Irregular	Entire Undolate	Raised Flat	Punctiform/Medium Large	Smooth Smooth	Shiny Cloudy	White/Yellow White	Opaque Translucent
	10^{-8}	1	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
		2	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
		3	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque

Table 5: Morphological Examination of Bacterial Colonies in Green Salad served at Public Restaurants

Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
GS	10^{-6}	1	Circular	Entire	Flat	Punctiform Large	Smooth	Shiny	White	Opaque
		2	Circular	Entire	Flat	Punctiform	Smooth	Shiny	White	Opaque
		3	Circular	Entire	Flat	Punctiform Medium	Smooth	Shiny	White	Opaque
	10^{-7}	1	Circular	Entire	Flat	Punctiform	Smooth	Shiny	White	Opaque
		2	Circular	Entire	Flat	Punctiform	Smooth	Shiny	White	Opaque
		3	Circular	Entire	Flat	Punctiform	Smooth	Shiny	White	Opaque
	10^{-8}	1	Circular	Entire	Flat	Small and Large	Smooth	Shiny	White	Opaque
		2	Circular	Entire	Flat	Small and Large	Smooth	Shiny	White	Opaque
		3	Circular	Entire	Flat	Small	Smooth	Shiny	White	Opaque

Table 6: Morphological Examination of Bacterial Colonies in Green Salad served at Fast Food Restaurants

Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
GS	10^{-6}	1	Circular	Entire	Flat	Punctiform Small Large	Smooth	Cloudy Shiny Cloudy	Crème Light Yellow Crème	Translucent Opaque Translucent
		2	Circular	Entire	Flat	Punctiform Small Large	Smooth	Cloudy Shiny Cloudy	Crème Light Yellow Crème	Translucent Opaque Translucent
		3	Circular	Entire	Flat	Punctiform Small Large	Smooth	Cloudy Shiny Cloudy	Crème Light Yellow Crème	Translucent Opaque Translucent
	10^{-7}	1	Circular Rhizoid	Entire Undolate	Flat	Punctiform Large	Smooth Smooth	Dull Cloudy	Crème	Translucent

		2	Circular Rhizoid	Entire Undolate	Flat	Punctiform Large	Smooth Smooth	Dull Cloudy	Crème	Translucent
		3	Circular Rhizoid	Entire Undolate	Flat	Punctiform Large	Smooth Smooth	Dull Cloudy	Crème	Translucent
	10^{-8}	1	Circular Rhizoid	Entire Rhizoid	Flat Flat	Punctiform Large	Smooth	Dull Cloudy	White	Translucent
		2	Circular Rhizoid	Entire Rhizoid	Flat Flat	Punctiform Large	Smooth	Dull Cloudy	White	Translucent
		3	Circular Rhizoid	Entire Rhizoid	Flat Flat	Punctiform Large	Smooth	Dull Cloudy	White	Translucent

a) Preparation of Fungal Smears

The lactophenol cotton blue (LPCB) wet mounts were prepared for observing fungi isolated from
the agar plates after incubation. The pure fungal cultures so obtained were mounted on slides for further examination.

Gram staining of bacterial smears

The bacteria were first stained with crystal violet followed by a brief treatment with Gram's iodine. The iodine functions as a mordant to help the crystal violet bind more firmly. The bacteria were then rinsed with ethanol. Gram positive bacteria, which have multiple layers of peptidoglycan, retained the crystal violet while it was quickly rinsed out of Gram negative bacteria because their peptidoglycan is a single layer thick. The bacteria were stained a second time (counter stained) with the dye safranin which have not shown up on the already purple Gram positive but have stained the decolorized Gram negative bacteria red.

Microscopic examination of the slides

The bacterial as well as fungal mounts prepared were examined under microscope for their identification and the results are presented in plates 19 to 24.

a) Bacterial and Fungal Smears under Microscope

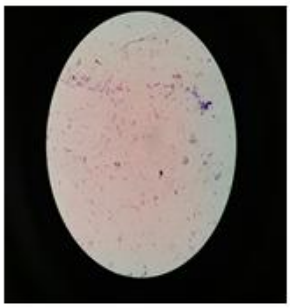

Plate 19: A gram stain of *E. coli* (gram-negative rods, in red) in smear of Green Salad served at Private Restaurants

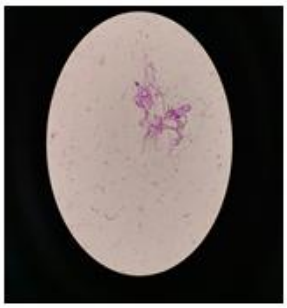

Plate 20: A mixed gram stain of *B. subtilis* (gram-positive rods in chains, in purple) and *B. cereus* (gram-positive rods present singly or in pairs, in purple) in smear of Green Salad served at Public Restaurants

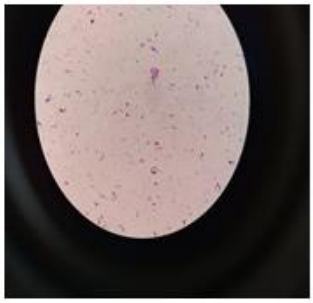

Plate 21: A gram stain of *S. aureus* (gram-positive cocci in cluster, in purple) in smear of Green Salad served at Fast Food Restaurants

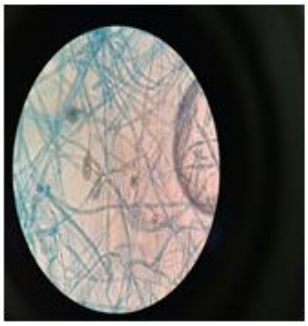

Plate 22: *Aspergillus* in smear of Green Salad served at Private Private Restaurants

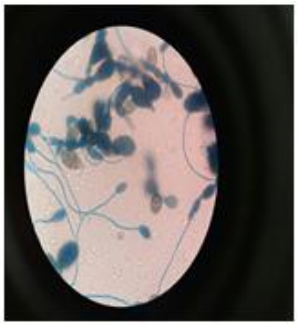

Plate 23: *Aspergillus* in smear of Green Salad served at Public Public Restaurants

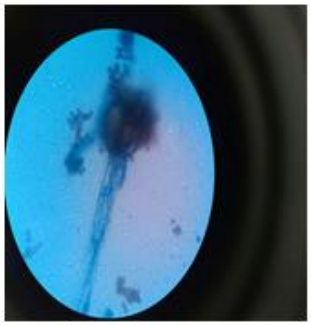

Plate 24: *Penicillium* in smear of Green Salad served at Fast Food Restaurants

b) Identification of Pathogenic Microbes

After the microscopic examination of these colonies, they were again grown on differential media in order to confirm them. After specified incubation periods, the microbes confirmed from the various samples are enlisted in the Table 7.

Table 7: Microbes Identified Green Salad served at Selected Restaurants

S. No.	Food Sample	Type of Restaurant	Microbes isolated from Samples
1	Green Salad	Private	*E. coli, Staphylococcus aureus, Aspergillus, Penicillium*
2	Green Salad	Public	*Bacillus subtilis, Bacillus cereus, Aspergillus*
3	Green Salad	Fast Food	*Bacillus subtilis, Staphylococcus aureus, Penicillium*

Conclusion

Green salad samples from all three types of restaurants had shown high incidence of total viable counts. The highest bacterial CFU/ml in samples of green salad was however recorded as 7.6×10^{10} at 10^{-8} dilution in private restaurants followed by a mean CFU/ml value of 5.7×10^{10} and 4.0×10^{10} respectively in case of public and fast food restaurants at the same dilution. The pathogens found in samples of green salad from private restaurants include *E.coli, Staphylococcus aureus, Aspergillus, Penicillium* while those from public establishments had *Bacillus subtilis, Bacillus cereus, Aspergillus, Yeast* (Tables 4.3.3.4 and 4.3.3.5). However, *Bacillus subtilis, Staphylococcus aureus, Penicillium* were detected in case of fast food restaurants (Table 4.3.3.6). The p-values between private versus public, private versus fast food and public versus fast food restaurants did not favour significance in microbial colonies. Hence, it could be concluded that all the three types of restaurants serve green salad with similar microbiological quality. This perhaps may be due the reason that salad being highly perishable is very difficult to be protected from microbial contamination. Thus, green salad has been reported responsible in maximum of the eating out food poisoning outbreaks.

References

1. Abu-Ruwaida AS, Sawaya WN, Dashti BH, Murard M, Al-Othman HA. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. Journal of Food Protection. 1994; 57:887-892.
2. Adwan G, Shanab BA, Adwan K. Enterotoxigenic *Staphylococcus aureus* in raw milk in the North of Palestine, Turkish Journal Biology. 2005; 29:229-232.
3. Barro Nicolas, Bello Abdoul R, Savadogo Aly, Ouattara Cheik Amadou T, Ilboudo Jules A, Traore Alfred S. Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso), African Journal of Biotechnology. 2006; 5(II):1107-1112.
4. Busani L, Scavia G, Luzzi I, Caprioli A. Laboratory surveillance for prevention and control of foodborne zoonoses, Annali dell'Istituto Superiore di Sanita. 2006; 42:401-404.
5. Capita R, Alonso-Calleja C, Garcia-Arias MT, Moreno B, Del Camino Garcia-Fernandez M. Methods to detect the occurrence of various indicator bacteria on the surface of retail poultry in Spain. Journal of Food Science. 2002; 67:765-771.
6. Centers for Disease Control and Prevention (CDC) Surveillance for Creutzfeldt-Jakob disease, United States, MMWR Morb Mortal Wkly Rep. 1996; 45:665-668.
7. Centers for Disease Control and Prevention Annual Report. CDC/USDA/FDA foodborne diseases active surveillance network. CDC's Emerging Infection Program, 2000.
8. Christison CA, Lindsay D, Holy Von A. Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg. South Africa Food Control. 2008; 19(7):727-733.
9. Jay JM. Modern Food Microbiology, 6th edition. Aspen Publishers, Maryland, 2006.
10. Kampen JV, Gross R, Schultink W, Usfar A. The Microbiological Quality of Street Foods in Jakarta as Compared to Home prepared foods and foods from tourist hotels. International Journal of Food Sciences and Nutrition. 1998; 49: 17-26.
11. Nazni P, Jaganathan A. Study on Microbial Analysis of Street-Vended Food Samples sold in Salem District. International Journal of Research in Biological Sciences. 2014; 4(3):75-78.
12. Pereira K, Schmidt F, Guaraldo A, Franco R, Dias V, Passos L. Disease: as a foodborne illness. Journal Food Protection. 2009; 72:441-6.
13. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL. Foodborne illness acquired in the United States - major pathogens. Journal Emergency Infection Disease. 2011; 17:7-15.