International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 6; November 2017; Page No. 35-44

Examination of microbiological quality of karahi paneer served at selected restaurants

[*1] Prabhjot Kaur, [2] Dr. Vinti Davar, [3] Dr. Neelam
[1] Assistant Professor, Department of Home Science, GNG College, Yamunanagar, Haryana, India
[2] Professor (Retd.) cum Ex-Chairperson, Department of Home Science, Kurukshetra University, Kurukshetra, Haryana, India
[3] Professor cum Chairperson, Department of Microbiology, Kurukshetra University, Kurukshetra, Haryana, India

Abstract
Food quality has always been a challenge for all the restaurateurs. 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. The microbiologically safe levels are generally not met by the food served in the restaurants thus leading to a number of food poisoning outbreaks. On microbiological analysis, karahi paneer samples showed higher viable counts after microbiological examination. The maximum number of bacterial (6.57×10^{10} CFUs per ml.) and fungal (1.3×10^{6} CFUs per ml.) colonies were found to be grown at 10^{-8} dilution of the sample of karahi paneer obtained from the public restaurants. *Bacillus cereus, E.coli, Aspergillus* were found in karahi paneer of private restaurants while *Bacillus cereus, Pseudomonas aeruginosa, Aspergillus, Rhizopus* were found in unsafe levels in samples from public restaurants. Karahi paneer samples of fast food restaurants had *Staphylococcus aureus* and *Aspergillus* only. The results of t-test marked a highly significant difference in bacterial and fungal colonies in private and public units. However, no significant difference was observed between private versus fast food restaurants. But the levels of pathogens present in all samples were highly unacceptable. This could be associated with maximum number of reported food poisoning outbreaks related to paneer.

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

Introduction
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.

Objectives
The research was carried out with the following objectives:
- To assess the microbiological adequacy of karahi paneer served at the selected restaurants
- To identify the pathogens present in karahi paneer served at the studied restaurants

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) [14]. These pathogens, though, usually cause self-limiting gastroenteritis, complications may occur, resulting in more severity (Zhao *et al*, 2001) [18]. *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 diarrhoea. 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). 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). Thus, assessment of the chemical quality of these food products is very important to improve health of consumers (Jay, 2006). 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 study was conducted to isolate and identify bacterial pathogens/ contaminants in paneer samples sold in Chandigarh. Fifty eight samples of paneer bought at random were cultured on several media. Bacterial colony counts were also done. The predominant organisms isolated were Staphylococcus species, aerobic spore bearers, *Klebsiella pneumoniae*. *Campylobacter jejuni*, *Acinetobacter* species and *Streptococcus* species. The viable bacterial counts obtained ranged from 3 x 102 to 9.7 x 1010 cfu/ml (Vaishnavi *et al* ., 2001). Pasteurized and raw milk that had been inoculated with 104 cfu/ml of *Escherichia coli* O157:H7 culture, were used for manufacturing paneer samples which were vacuum-packaged and storcd at 4°C, 8°C, and 28°C. Survival and growth of *Escherichia coli* O157:H7 in paneer samples was determined after every 4 h for up to 48 h. *Escherichia coli* O157:H7 could survive the manufacturing process of paneer and were present at the end of the storage period (at 28°C) in significantly ($p < 0.05$) greater numbers than the initial inoculum. No significant difference in survival and growth were noticeable in paneer samples manufactured from raw milk. Refrigeration (4°C or 8°C) effectively inhibits the growth of *Escherichia coli* O157: H7, but this pathogen can survive over a period of 48 hours. Our observations suggest that unpasteurized or improperly pasteurized milk could be an important source for transmission of *Escherichia coli* O157:H7 through paneer and appropriate steps must be taken by government agencies to ensure consumer safety for this dairy product (Sidhi *et al*, 2006).

Aerobacter aerogenes was the main coliform organism recovered from the frozen green beans (Raccach *et al*., 2007). Seventeen isolates were characterized from the samples on PCA with percentage of occurrence of different microorganisms characterized as follows: *Bacillus cereus* (29.4 per cent), *Enterobacter aerogenes* (29.4 per cent), *Salmonella* spp. (17.6 per cent), *Flavobacterium* spp. (11.8 per cent), *Micrococcus* spp. (5.9 per cent), and *Staphylococcus* auerus (5.9 per cent) (Okonko *et al*., 2008). In 2008 Byrne *et al*., in his studies showed similar results in a meat industry. Report in journal indicates that the highest total viable count was observed in the cooking area, with 133 colony forming units per cubic metre (cfu/ml), blast chill area had highest coliform counts (8 cfu/ml) while *Staphylococcus aureus* counts were highest in preparation areas (8 cfu/ml) (Byrne *et al*., 2008).

The majority of food poisoning outbreaks are 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 per cent) and *Listeria monocytogenes* (4 per cent) 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 per cent of E1 washing waters were very impure, while, 44.5 per cent of second washing waters (E2) were impure, 44.5 per cent very impure and 11 per cent acceptable. 45.45 per cent of E3 washing waters were acceptable, 27.27 per cent impure and 27.27 per cent 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 (8.6×10^5 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).

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 karahi paneer 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).

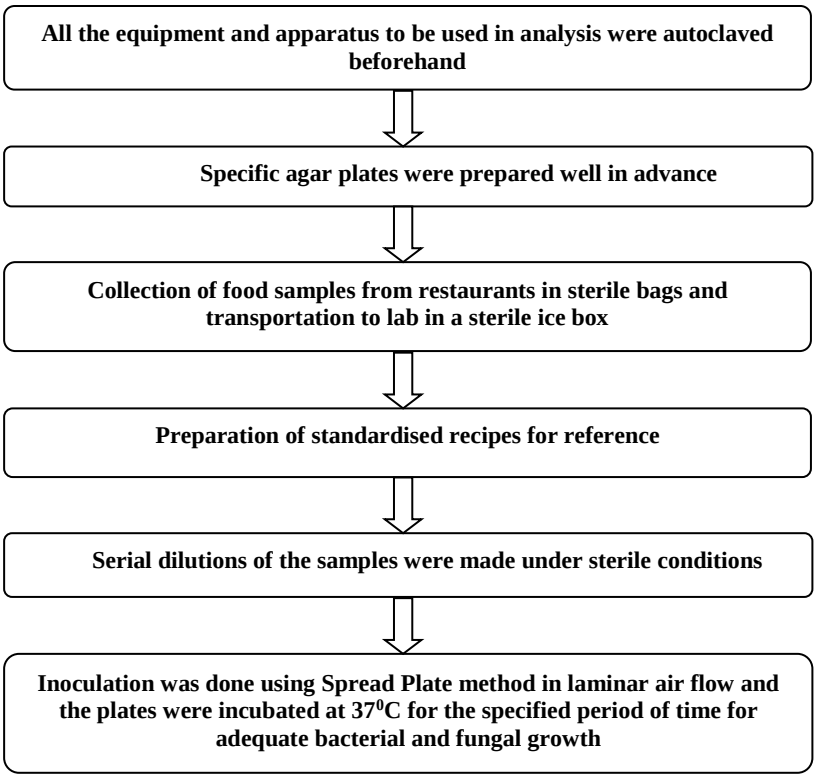

Fig 1: Methodology for Microbiological Analysis of the Selected Recipes at a Glance

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 37^0 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.

Results and Discussion

The karahi paneer samples collected from private, public and fast food restaurants were examined using standard microbiological procedure for any bacterial and fungal growth. The table 1 demonstrated the mean of bacterial and fungal colony forming units (CFUs/ml) calculated after observing the incubated agar plates of the studied samples of karahi paneer.

Table 1: Comparison of Microbial Flora (CFU/ml) of Karahi Paneer (KP) served in Private, Public and Fast Food Restaurants

KP	Dilution	Mean Bacterial CFU**	Bacterial CFU**/ml	Dilution	Mean Fungal CFU**	Fungal CFU**/ml
R1	10^{-6}	232	2.32×10^9	10^{-2}	26	2.6×10^3
	10^{-7}	156	1.56×10^{10}	10^{-3}	15	1.5×10^4
	10^{-8}	37	3.7×10^{10}	10^{-4}	5	5×10^5
R2	10^{-6}	248	2.48×10^9	10^{-2}	30	3.0×10^4
	10^{-7}	174	1.74×10^{10}	10^{-3}	23	2.3×10^5
	10^{-8}	65	6.5×10^{10}	10^{-4}	13	1.3×10^6
R3	10^{-6}	151	1.51×10^9	10^{-2}	20	2.0×10^4
	10^{-7}	81	8.1×10^9	10^{-3}	12	1.2×10^5
	10^{-8}	44	4.4×10^{10}	10^{-4}	6	6×10^5
SR*	10^{-6}	20	2.0×10^8	10^{-2}	2	2×10^3
	10^{-7}	15	1.5×10^9	10^{-3}	1	1×10^4
	10^{-8}	8	8×10^9	10^{-4}	1	1×10^5

*Standardised Recipe ** Colony Forming Units

The mean scores for bacterial CFUs at 10^{-6} dilution in samples of karahi paneer are 232, 248 and 151 respectively for private, public and fast food restaurants while the mean for standardized recipe is 20 only. Private, public and fast food restaurants have shown 156, 174 and 81 mean bacterial colonies respectively at 10^{-7} dilution in comparison to 15 of the standardized recipe. The mean values for bacterial colonies in the samples of karahi paneer collected from private public and fast food restaurants has been 37, 65 and 44 respectively at 10^{-8} dilution. All of the studied samples have

crossed the mean value of only 8 by the standardized recipe. The mean fungal colonies shown by the tested samples are 26, 15 and 5 in the karahi paneer of private restaurants at 10^{-2}, 10^{-3} and 10^{-4} dilutions respectively. The mean scores for fungal colonies in samples of karahi paneer served in public restaurants are 30, 23 and 13 at 10^{-2}, 10^{-3} and 10^{-4} dilutions respectively. However, fast food restaurants have been recorded 20, 12 and 6 mean fungal colonies in karahi paneer samples at the same dilutions. Mean fungal scores obtained by the standardized recipe are 2, 1 and 1 respectively at 10^{-2}, 10^{-3} and 10^{-4} dilutions. It is hereby noted that microbiological quality of karahi paneer served in private, public and fast food restaurants was expectedly deteriorated. The maximum number of bacterial (6.57×10^{10} CFUs per ml.) and fungal (1.3×10^{6} CFUs per ml.) colonies were found to be grown at 10^{-8} dilution of sample of karahi paneer obtained from public restaurants. However, not much difference was noted even in karahi paneer samples of private as well as fast food restaurants. The standardized recipe prepared under sterile conditions has shown fewer microbial colonies as compared to samples of the studied restaurants.

Table 2: Comparison of Bacterial CFUs in Karahi Paneer served in Private, Public and Fast Food Restaurants

t-test		Type of Restaurants	t	df	Sig. (2-tailed)
CFU	Equal variances assumed	Private versus Public	- 6.193	16	.000**
	Equal variances not assumed		- 6.193	15.999	.000**
CFU	Equal variances assumed	Private versus Fast Food	.547	16	.592
	Equal variances not assumed		.547	15.962	.592
CFU	Equal variances assumed	Public versus Fast Food	6.886	16	.000**
	Equal variances not assumed		6.886	15.950	.000**

** Significant at $p \leq 0.001$

The table 2 depicts the outcome of t-test carried out on private versus public restaurants, private versus fast food restaurants and public versus fast food restaurants. The results mark a highly significant difference in bacterial colonies of samples of karahi paneer as indicated by the p-value of 0.000 at 99 per cent confidence level. However, no significant difference was observed with a p-value of 0.592 at the same level of confidence between bacterial colonies in karahi paneer served in private as well as fast food restaurants suggesting a similar bacteriological growth in karahi paneer served there. The researcher thus suggests that public sector restaurants should try hard to pay more attention towards the prevention of bacteriological spoilage of paneer thus reducing the chances of food poisoning.

Plate 1: Bacterial Colonies on NA Plate of Karahi Paneer of Private Restaurants (10^{-6} Dilution)

Plate 2: Bacterial Colonies on NA Plate of Karahi Paneer of Private Restaurants (10^{-7} Dilution)

Plate 3: Bacterial Colonies on NA Plate of Karahi Paneer of Private Restaurants (10^{-8} Dilution)

Plate 4: Bacterial Colonies on NA Plate of Karahi Paneer of Public Restaurants (10^{-6} Dilution)

Plate 5: Bacterial Colonies on NA Plate of Karahi Paneer of Public Restaurants (10^{-7} Dilution)

Plate 6: Bacterial Colonies on NA Plate of Karahi Paneer of Public Restaurants (10^{-8} Dilution)

Plate 7: Bacterial Colonies on NA Plate of Karahi Paneer of Fast Food Restaurants (10^{-6} Dilution)

Plate 8: Bacterial Colonies on NA Plate of Karahi Paneer of Fast Food Restaurants (10^{-7} Dilution)

Plate 9: Bacterial Colonies on NA Plate of Karahi Paneer of Fast Food Restaurants (10^{-8} Dilution)

Plates 1 to 9 above are agar plates of bacterial colonies grown in various samples of karahi paneer from the selected restaurants at the specified dilutions.

Table 3: Comparison of Fungal CFUs in Karahi Paneer served in Private, Public and Fast Food Restaurants

t-test		Type of Restaurants	t	df	Sig. (2-tailed)
CFU	Equal variances assumed	Private versus Public	- 1.773	16	.095
	Equal variances not assumed		- 1.773	14.767	.097
CFU	Equal variances assumed	**Private versus Fast Food**	.514	16	.614
	Equal variances not assumed		.514	13.237	.616
CFU	Equal variances assumed	Public versus Fast Food	2.923	16	.010*
	Equal variances not assumed		2.923	15.423	.010*

* Significant at $p \leq 0.05$

It was evident from table 3 that there exists a significant difference in fungal CFUs per ml. in the samples of karahi paneer taken from private and fast food restaurants. The p-value of 0.010 at 95 per cent confidence level marks the significant difference in public versus fast food restaurants of this study. But not any significance has been observed in private versus public and private versus fast food restaurants according to outcomes of t-test with p-values of 0.095 and 0.614 at the same confidence level. This reveals that same favouring conditions for the growth of fungus are present in samples of karahi paneer from restaurants of all the three types.

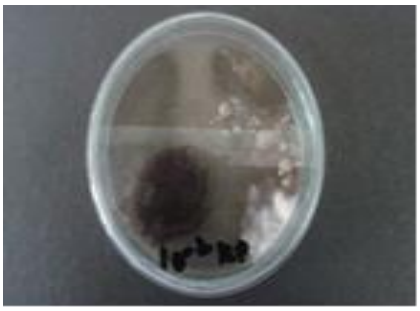

Plate 10: Fungal Colonies on CYGA Plate of Karahi Paneer served at Private Restaurants (10^{-2} Dilution)

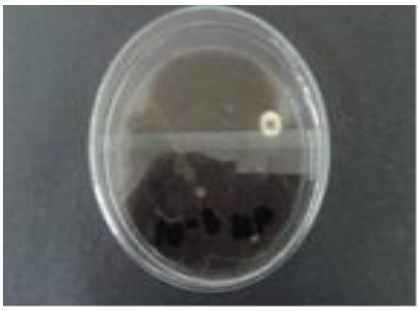

Plate 11: Fungal Colonies on CYGA Plate of Karahi Paneer served at Private Restaurants (10^{-3} Dilution)

Plate 12: Fungal Colonies on CYGA Plate of Karahi Paneer served at Private Restaurants (10^{-4} Dilution)

Plate 13: Fungal Colonies on CYGA Plate of Karahi Paneer served at Public Restaurants (10^{-2} Dilution)

Plate 14: Fungal Colonies on CYGA Plate of Karahi Paneer served at Public Restaurants (10^{-3} Dilution)

Plate 15: Fungal Colonies on CYGA Plate of Karahi Paneer served at Public Restaurants (10^{-4} Dilution)

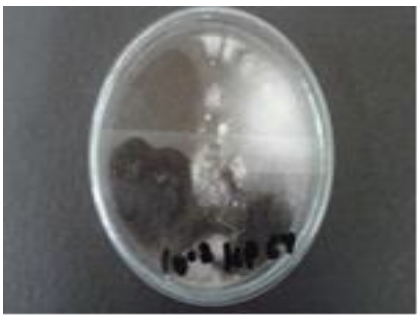

Plate 16: Fungal Colonies on CYGA Plate of Karahi Paneer served at Fast Food Restaurants (10^{-2} Dilution**)**

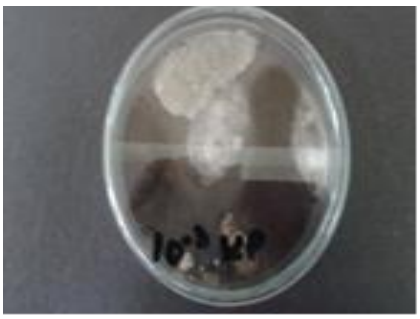

Plate 17: Fungal Colonies on CYGA Plate of Karahi Paneer served at Fast Food Restaurants

Plate 18: Fungal Colonies on CYGA Plate of Karahi Paneer served at Fast Food Restaurants

Plates 10 to 18 depict fungal colonies of samples of karahi paneer collected from private, public and fast food restaurants. These plates were obtained on CYG agar after the incubation period of 4 to 5 days at 37^0 C.

Microbiological Adequacy of the Selected Recipes

The isolated bacterial colonies obtained on agar plates were then transferred to nutrient broth tubes for incubation at standard conditions (Photo 1) and were then preserved for further proceedings. The isolated fungal colonies were however cut and transferred again on fresh agar plates for substantial growth at optimum conditions.

Morphological Examination of the Microbial Colonies

Tables 4 to 6 depict results of morphological features examined by the researcher to help in identification of microbes found on various plates of the studied samples of karahi paneer from private, public and fast food restaurants.

Table 4: Morphological Examination of Bacterial Colonies in Karahi Paneer (KP) served at Private Restaurants

S. No.	Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
1.	KP	10^{-6}	1	Irregular	Undolate	Flat	Medium	Smooth	Shiny	Crème	Translucent
			2	Circular Circular	Entire Entire	Flat Raised	Punctiform Medium	Smooth Smooth	Shiny Waxy	Yellow Light Creme	Opaque Translucent
			3	Circular	Entire	Raised	Medium	Smooth	Waxy	Light Creme	Translucent
		10^{-7}	1	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
			2	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
			3	Irregular	Undolate	Flat	Medium	Smooth	Shiny	Crème	Translucent
		10^{-8}	1	Irregular	Undolate	Raised	Medium	Smooth	Shiny	Buff	Translucent
			2	Filamentous	Lobate	Flat	Large	Smooth	Waxy	Crème	Translucent
			3	Filamentous	Lobate	Flat	Large	Smooth	Waxy	Crème	Translucent

Table 5: Morphological Examination of Bacterial Colonies in Karahi Paneer (KP) served at Public Restaurants

S. No.	Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
1.	KP	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	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^{-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 6: Morphological Examination of Bacterial Colonies in Karahi Paneer (KP) served at Fast Food Restaurants

S. No.	Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
1.	KP	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	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^{-8}	1	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque
			2	Circular Circular	Entire Entire	Raised Flat	Punctiform Small	Smooth Smooth	Shiny Dull	Bright Yellow Crème	Opaque Translucent
			3	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Bright Yellow	Opaque

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.

b) 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

a) Bacterial and Fungal Smears

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.

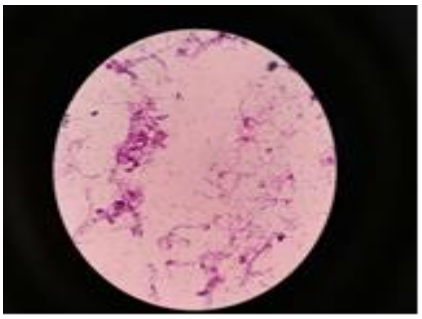

Plate 19: A Gram stain of mixed *Bacillus cereus* (gram-positiverods in purple) and *E. coli* (gram - negative rods, in red) in smear of Karahi Paneer served at Private Restaurants

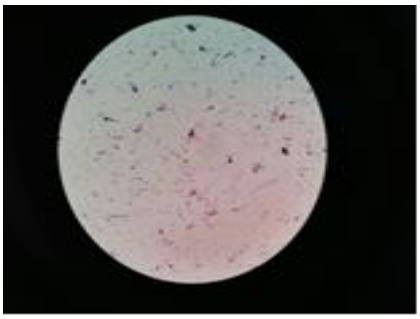

Plate 20: A Gram stain of mixed *Bacillus cereus* (gram-positiverods in purple) and *E. coli* (gram - negative rods, in red) in smear of Karahi Paneer served at Public Restaurants

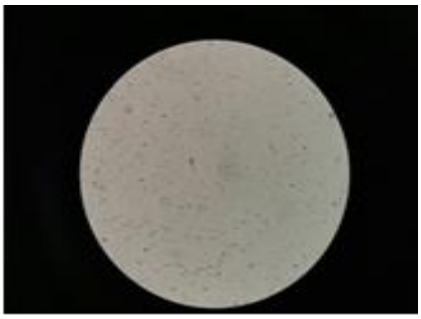

Plate 21: A Gram stain of mixed *Bacillus cereus* (gram-positiverods in purple) and *E. coli* (gram - negative rods, in red) in smear of Karahi Paneer served at Fast Food Restaurants

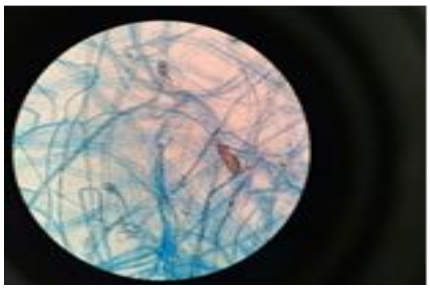

Plate 22: *Aspergillus* in smear of Karahi Paneer served at Private Restaurants

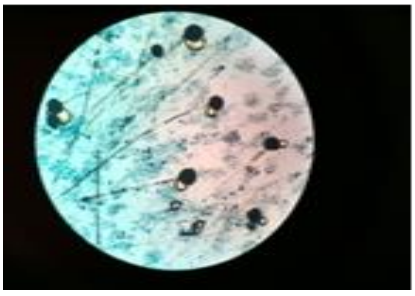

Plate 23: *Aspergillus* in smear of Karahi Paneer served at Public Restaurants Restaurants

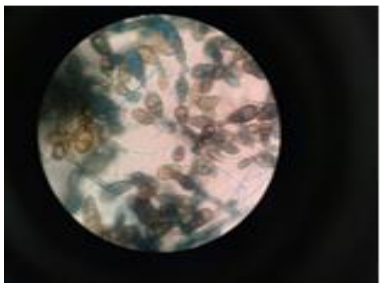

Plate 24: *Aspergillus* in smear of Karahi Paneer served at Fast Food Restaurants

b) Identification of Pathogenic Microbes

After 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 various samples of karahi paneer are enlisted in the Table 7.

Table 7: Microbes Identified in the Selected Recipes served in Private Restaurants

S. No.	Food Sample	Type of Restaurant	Microbes isolated from Samples
1	Karahi Paneer	Private	*Bacillus cereus, E.coli, Aspergillus*
2	Karahi Paneer	Public	*Bacillus cereus, Pseudomonas aeruginosa, Aspergillus, Rhizopus*
3	Karahi Paneer	Fast Food	*Staphylococcus aureus, Aspergillus*

Conclusion

Karahi paneer samples also showed higher viable counts after microbiological examination. The maximum number of bacterial (6.57×10^{10} CFUs per ml.) and fungal (1.3×10^{6} CFUs per ml.) colonies were found to be grown at 10^{-8} dilution of the sample of karahi paneer obtained from the public restaurants. *Bacillus cereus, E.coli, Aspergillus* were found in karahi paneer of private restaurants while *Bacillus cereus, Pseudomonas aeruginosa, Aspergillus, Rhizopus* were present in unsafe levels in samples from public restaurants (Table 7). Karahi paneer samples of fast food restaurants had *Staphylococcus aureus, Aspergillus* only. The results of t-test marked a highly significant difference in the bacterial and fungal colonies in private and public units. However, no significant difference was observed between private versus fast food restaurants. But the levels of pathogens present in all the samples were highly unacceptable. This could be associated with maximum number of reported food poisoning outbreaks related to paneer. The microbes detected in karahi paneer are in accordance with a study conducted to isolate and identify bacterial pathogens or contaminants in paneer samples sold in Chandigarh wherein fifty eight samples of paneer bought at random were cultured on several media. Bacterial colony counts were also done. The predominant organisms isolated were *Staphylococcus* species, aerobic spore bearers, *Klebsiella pneumuoniae*, *Campylobacter jejuni*, *Acinetobacter* species and *Streptococcus* species. The viable bacterial counts obtained ranged from 3×10^{2} to 9.7×10^{10} CFUs / ml. (Vaishnavi *et al*, 2001).

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 A Jules and 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. Byrne B, Lyng J, Dunne G, Bolton OJ. An assessment of the microbial quality of the air within a pork processing plant, Food Control (Elsevier). 2008; 19(9):915-920.
6. 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.
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 A von. 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. Okonko, Iheanyi Omezuruike, Ogunjobi, Adeniyi Adewale, Fajobi, Enobong Aloysius, *et al.* Comparative studies and microbial risk assessment of different Ready-to-Eat (RTE) frozen sea-foods processed in Ijora-olopa, Lagos State, Nigeria, 2008.
13. Pereira K, Schmidt F, Guaraldo A, Franco R, Dias V, Passos L. Disease: as a foodborne illness. Journal Food Protection. 2009; 72:441-6.
14. Raccach M, Juvcn B, Henis Y. Variations in bacterial counts during the production of frozen green beans, International Journal of Food Science and Technology. 2007; 7(4):417-421.
15. 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.
16. Sidhi Wahi, Sunita Bansal, Moushumi Ghosh, and Abhijit Ganguli. Growth and Survival of Escherichia coli O157:H7 during Manufacture and Storage of Indian Cheese (Paneer). Foodborne Pathogens and Disease. 2006; 3(2):184-189.

17. Vaishnavi C, Singh S, Grover R, Singh K. Bacteriological study of Indian cheese (paneer) sold in Chandigarh. Ind. J of Med. Microbiology. 2001; 19:224-226.
18. Zhao C, Ge B, Villena JD, Sudler R, Yeh E. Prevalence of Campylobacter spp., Escherichia coli and Salmonella serovars in retail chicken, Turkey, pork and beef from the Greater Washington D.C., Area. Applied Environ. Microbiol. 2001; 67:5431-5436.