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Examination of microbiological quality of dal makhani served at selected restaurants

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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. The total viable count of dal makhani samples was found to be higher as compared to the standardized recipe. The highest level of bacterial and fungal colonies with a mean value of 8.5×10^{10} CFU/ml and 4×10^{5} CFU/ml respectively were found in dal makhani procured from public restaurants. The pathogens found in dal makhani of private restaurants include *Bacillus subtilis, Bacillus cereus, E. coli, Penicillum* while that of public sector had *Bacillus cereus, Staphylococcus aureus, E. coli, Salmonella, Aspergillus*. However, dal makhani samples of fast food restaurants carried *Bacillus cereus, E. coli* in unacceptable amounts. The results of t-test highlighted the microbiological inadequacy of dal makhani served in public sector restaurants. The presence of unsafe levels of pathogenic microbes proved that contaminated food was being served in public units. No fungal contamination was however found in dal makhani of fast food restaurants and the standardized recipe.

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 socializing. 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 evaluate the microbiological adequacy of dal makhani served at the selected restaurants
- To identify the pathogens present in dal makhani 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 zoometric (Busoni *et al.*, 2006) [2]. These pathogens, though, usually cause self-limiting gastroenteritis, complications may occur, resulting in more severity (Zhao *et al.*, 2001) [11]. *Staphylococcus aureus* is one of the most common agents in bacterial food poisoning outbreaks (Adwa *et al.*, 2005) [1] 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. Thus, assessment of the chemical quality of these food products is very important to improve health of consumers (Jay, 2006) [6]. 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.

Aerobacter aerogenes was the main coliform organism recovered from the frozen green beans (Raccach *et al.*, 2007) [10]. 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) [9]. 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).

A majority of food poisoning outbreaks is associated with improper holding that occurs in institutional settings (CDC 1996, 2000) [4]. 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*. 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 x 10^5 cfu/knife) such as coliforms, *Staphylococcus aureus*, *Salmonella* and *Shigella*. 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 Autoclaved Agar plates
Nutrient Media

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.

Method

Serial dilutions of food samples were prepared in already autoclaved water blanks (Photo 1). Inoculation of autoclaved agar plates was carried out by spread plate method. Microbiological quality examination of dal makhani 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.

Photo 1: Serial Dilutions of Dal Makhani

Photo 2: Studying the Colony Morphology of Bacterial Agar Plates

The inoculation of collected samples was done in triplicates on Nutrient Agar (NA) for bacterial colonies and Potato Dextrose Agar (PDA) 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).

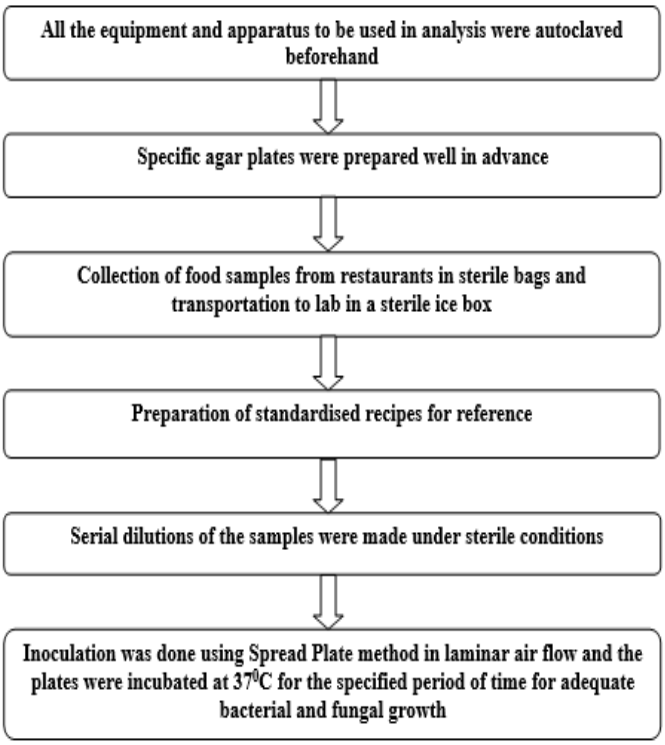

Fig 1: Methodology for Microbiological Analysis of Dal Makhani at a Glance

Results and Discussion

The present data comprised of total 6 restaurants: two private restaurants (R1), two public restaurants (R2) and two fast food restaurants (R3). Dal makhani samples were obtained as per inspection plan made and were microbiologically analyzed by using standard methods like spread plate, counting CFUs, isolation of bacteria and fungus, physical examination of the microbial colonies, preparation of smears and microscopic examination of the slides. In addition to this, the standardized recipe was formulated in consultation with chefs of different studied outlets. It was prepared by the researcher in hygienic settings and was also checked for pathogenic microbial growth. Thus, a total 7 samples were analyzed microbiologically for studying microbial flora in dal makhani served in private, public and fast food restaurants.

Microbiological Examination of Dal Makhani

The samples of dal makhani procured from private, public and fast food restaurants were examined for various microbial flora using standard microbiological procedures and results are expressed in Table 1 as well as plates and discussed thereafter.

Table 1: Comparison of Microbial Flora (CFU/ml) of Dal Makhani (DM) served in Private, Public and Fast Food Restaurants

DM	Dilution	Mean Bacterial CFU**	Bacterial CFU**/ml	Dilution	Mean Fungal CFU**	Fungal CFU**/ml
R1	10^{-6}	225	2.25×10^{9}	10^{-2}	1	1×10^{3}
	10^{-7}	104	1.04×10^{10}	10^{-3}	1	1×10^{4}
	10^{-8}	65	6.5×10^{10}	10^{-4}	1	1×10^{5}
R2	10^{-6}	230	2.33×10^{9}	10^{-2}	12	1.2×10^{4}
	10^{-7}	170	1.70×10^{10}	10^{-3}	8	8×10^{4}
	10^{-8}	85	8.5×10^{10}	10^{-4}	4	4×10^{5}
R3	10^{-6}	50	5.0×10^{8}	10^{-2}	0	0
	10^{-7}	40	4.0×10^{9}	10^{-3}	0	0
	10^{-8}	31	3.1×10^{10}	10^{-4}	0	0
SR*	10^{-6}	4	4×10^{7}	10^{-2}	0	0
	10^{-7}	2	2×10^{8}	10^{-3}	0	0
	10^{-8}	1	1×10^{9}	10^{-4}	0	0

*Standardised Recipe ** Colony Forming Units

The mean scores for bacterial CFUs at 10^{-6} dilution in samples of dal makhani are 225, 104 and 65 respectively for private, public and fast food restaurants while the mean for standardized recipe is 4 only. Private, public and fast food restaurants have shown 104, 170 and 40 mean colonies respectively at 10^{-7} dilution in comparison to 2 of the standardized recipe. The mean values for bacterial colonies in samples of dal makhani procured from private, public and fast food restaurants has been 65, 85 and 31 respectively at 10^{-8} dilution. All the studied samples have surpassed mean value of 1 shown by the standardized recipe. The mean fungal colonies shown by tested samples are 1, 1 and 1 in dal makhani of private restaurants at 10^{-2}, 10^{-3} and 10^{-4} dilutions respectively. The mean scores for fungal colonies in samples of dal makhani served in public restaurants are 12, 8 and 4 at 10^{-2}, 10^{-3} and 10^{-4} dilutions respectively. No fungal colonies have been observed in dal makhani samples of fast food restaurants as well as the standardized recipe thus no fungal contamination is found. The table 1 indicates that the highest level of bacterial and fungal colonies with a mean value of 8.5×10^{10} CFU/ml and 4×10^{5} CFU/ml respectively were found in the sample of dal makhani procured from public restaurants. This proves that contaminated food is being served in such units. The underlying factors may be less emphasis on cleanliness and hygiene by the employees or purchasing lower quality raw materials for preparing the dish. The incorporation of rotted or sub-standard vegetables (for tempering) used by the restaurants could also contribute to such results. No fungal contamination was however found in dal makhani of fast food restaurants and standardized recipe.

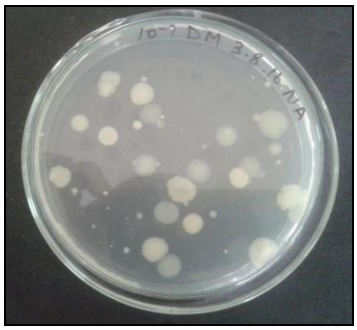

Plate 1: Bacterial Colonies on NA Plae of Dal Makhani Served at Private Restaurants (10^{-6} Dilution)

Plate 2: Bacterial Colonies on NA Plae of Dal Makhani Served at Private Restaurants (10^{-7} Dilution)

Plate 3: Bacterial Colonies on NA Plae of Dal Makhani Served at Private Restaurants (10^{-8} Dilution)

Plate 4: Bacterial Colonies on NA Plae of Dal Makhani Served at Public Restaurants (10^{-6} Dilution)

Plate 5: Bacterial Colonies on NA Plae of Dal Makhani Served at Public Restaurants (10^{-7} Dilution)

Plate 6: Bacterial Colonies on NA Plae of Dal Makhani Served at Public Restaurants (10^{-8} Dilution)

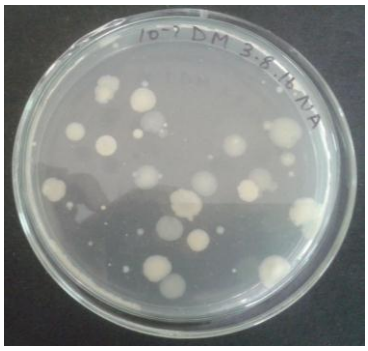

Plate 7: Bacterial Colonies on Plae of Dal Makhani Served at Fast Food Restaurants (10^{-6} Dilution)

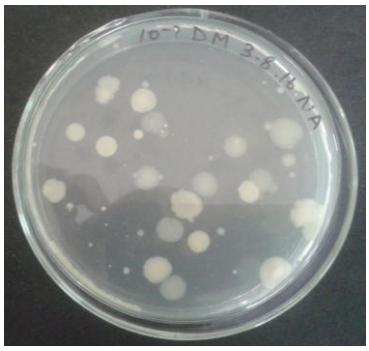

Plate 8: Bacterial Colonies on Plae of Dal Makhani Served at Fast Food Restaurants (10^{-7} Dilution)

Plate 9: Bacterial Colonies on Plae of Dal Makhani Served at Fast Food Restaurants (10^{-6} Dilution)

Plates 1 to 9 above depict the bacterial colonies in dal makhani of all the three types of restaurants at serial dilutions of 10^{-6}, 10^{-7} and 10^{-8}.

Table 2: Comparison of Bacterial CFUs in Dal Makhani 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	- 3.065	16	.007*
	Equal variances not assumed		- 3.065	15.824	.007*
CFU	Equal variances assumed	Private versus Fast Food	2.484	16	.024*
	Equal variances not assumed		2.484	8.015	.038*
CFU	Equal variances assumed	Public versus Fast Food	6.356	16	.000 **
	Equal variances not assumed		6.356	8.012	.000 **

*Significant at $p \leq 0.05$
**Significant at $p \leq 0.001$

In order to check significant level of difference in microbiological quality of dal makhani served in private, public and fast food restaurants, t-test was performed on private versus public restaurants, private versus fast food restaurants and public versus fast food restaurants. On analyzing the results of t-test performed on all three types of restaurants (Table 2), it was found that there exists a significant difference between bacterial colonies of private versus public as well as private versus fast food restaurants with p-values of 0.007 and 0.024 respectively at 95 per cent confidence level. However, difference between public versus fast food restaurants was noted to be highly significant with a p-value of 0.000 at 99 per cent confidence level. This highlighted the difference in microbiological quality of dal makhani being served in the studied establishments.

Plates 10 to 15 below depict the number of fungal colonies on various plates of dal makhani samples collected from private and public restaurants at the specified dilutions.

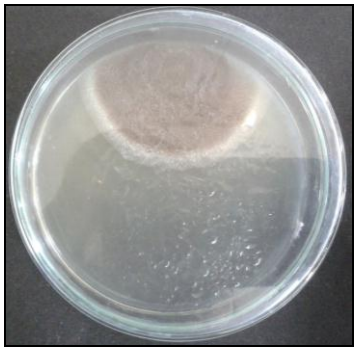

Plate 10: Fungal Colonies on PDA Plate of Dal Makhani served at Private Restaurants (10^{-2} Dilution)

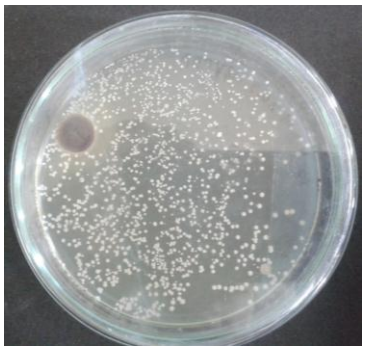

Plate 11: Fungal Colonies on PDA Plate of Dal Makhani served at Private Restaurants (10^{-3} Dilution)

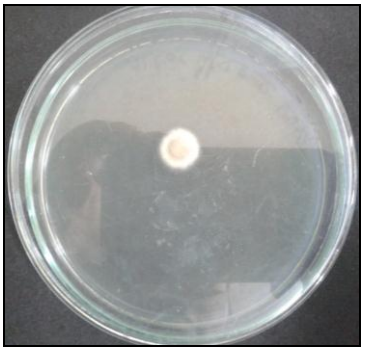

Plate 12: Fungal Colonies on PDA Plate of Dal Makhani served at Private Restaurants (10^{-4} Dilution)

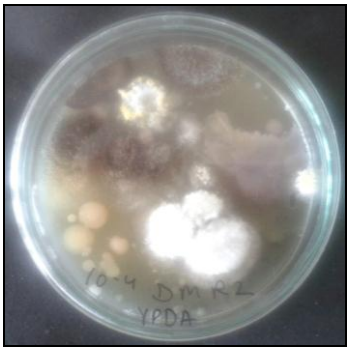

Plate 13: Fungal Colonies on PDA Plate of Dal Makhani served at Public Restaurants (10^{-2} Dilution)

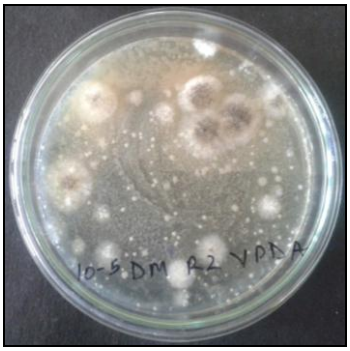

Plate 14: Fungal Colonies on PDA Plate of Dal Makhani served at Public Restaurants (10^{-3} Dilution)

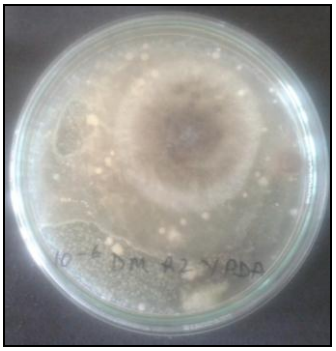

Plate 15: Fungal Colonies on PDA Plate of Dal Makhani served at Public Restaurants (10^{-4} Dilution)

The table 3 revealed the results of t-test conducted for fungal CFUs of dal makhani served in private, public and fast food restaurants. A highly significant difference has been observed between fungal colonies of private versus public restaurants and public versus fast food restaurants with a p-value of 0.000 at 99 per cent confidence level. However, t-test was unable to be computed between private versus fast food restaurants owing to same value of standard deviation. This highlighted the microbiological inadequacy of dal makhani served in public sector restaurants and the level of hygienic practices adopted by them thereby could be an important factor leading to food poisoning outbreaks. The researcher here opines that it is high time for such establishments to focus more on these sensitive and necessary issues in order to gain better patronage of the guests. The staff of public restaurants should be made aware and trained regarding HACCP procedures so that there is a reduction in number of food poisoning cases after eating out.

Table 3: Comparison of Fungal CFUs in Dal Makhani 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	- 6.062	16	.000**
	Equal variances not assumed		- 6.062	8.000	.000**
CFU	Equal variances assumed Equal variances not assumed	Private versus Fast Food	t cannot be computed as the standard deviations of bothgroups are zero		
CFU	Equal variances assumed	Public versus Fast Food	6.928	16	.000**
	Equal variances not assumed		6.928	8.000	.000**

** Significant at $p \leq 0.001$

Microbiological Adequacy of Dal Makhani

Tables 4 to 6 depict the results of morphological features examined by the researcher to help in the identification of microbes found on various plates of dal makhani from the studied 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.

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

Table 4: Morphological Examination of Bacterial Colonies in Dal Makhani served at Private Restaurants

S. No	Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical roperty
1.	DM	10^{-6}	1	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
			2	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
			3	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
		10^{-7}	1	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
			2	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
			3	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
		10^{-8}	1	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
			2	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent
			3	Circular	Entire	Flat	Punctiform	Smooth	Waxy	Crème	Translucent

Table 5: Morphological Examination of Bacterial Colonies in Dal Makhani served at Public Restaurants

S. No	Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
1.	DM	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 Dal Makhani served at Fast Food Restaurants

S. No	Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
1.	DM	10^{-6}	1	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
			2	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
			3	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
		10^{-7}	1	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
			2	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
			3	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
		10^{-8}	1	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
			2	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent
			3	Filamentous	Lobate	Flat	Medium	Smooth	Waxy	Crème	Translucent

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 16 to 21.

a) Bacterial and Fungal Smears under Microscope

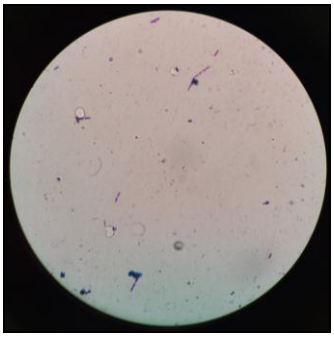

Plate 16: A gram stain of *Bacillus subtilis* (gram-positive rods with terminal endospores, in purple) in smear of Dal Makhani served at Private Restaurants

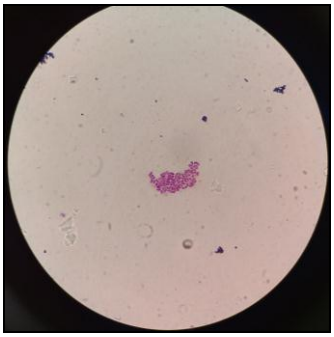

Plate 17: A gram stain of *S. aureus* (gram-positive cocci in cluster, in purple) in smear of Dal Makhani served at Public Restaurants

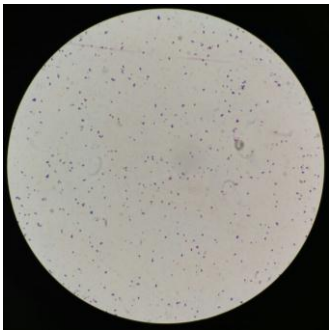

Plate 18: A gram stain of *Bacillus cereus* (gram-positive rods, in purple) in smear of Dal Makhani served at Fast Food Restaurants

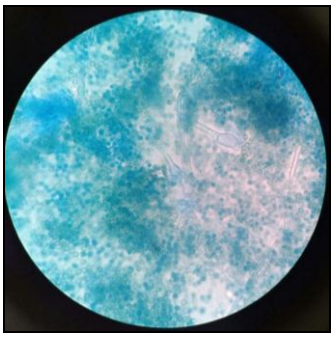

Plate 19: *Penicillium* in smear of Dal Makhani served at Private Restaurants

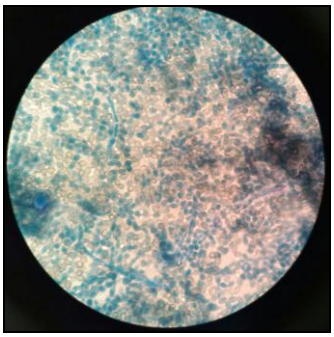

Plate 20: *Aspergillus* in smear of Dal Makhani served in Pubilc Restaurants

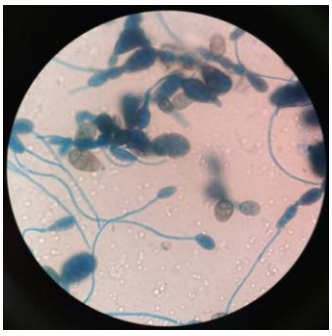

Plate 21: *Aspergillus* in smear of Dal Makhani served at Public Restaurants

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 are enlisted in Table 7.

Table 7: Microbes identified in Dal Makhani served at Selected Restaurants

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

Conclusion

The total viable count of dal makhani samples was found to be higher as compared to the standardized recipe. The highest level of bacterial and fungal colonies with a mean value of 8.5×10^{10} CFU/ml and 4×10^{5} CFU/ml respectively were found in dal makhani procured from public restaurants. The pathogens found in dal makhani of private restaurants include *Bacillus subtilis, Bacillus cereus, E. coli, Penicillum* while that of public sector had *Bacillus cereus, Staphylococcus aureus, E. coli, Salmonella, Aspergillus*. However, dal makhani samples of fast food restaurants carried *Bacillus cereus, E. coli* in unacceptable amounts. The results of t-test highlighted microbiological inadequacy of dal makhani served in public sector restaurants. The presence of unsafe levels of pathogenic microbes proved that contaminated food was being served in public units. No fungal contamination was however found in dal makhani of fast food restaurants and standardized recipe.

References

1. Adwa G, Shanab BA, Adwa K. Enterotoxigenic *Staphylococcus aureus* in raw milk in the North of Palestine, Turkish Journal Biology, 2005; 29:229-232.
2. Busoni 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.
3. 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.
4. Centers for Disease Control and Prevention. Annual Report. CDC/USDA/FDA foodborne diseases active surveillance network. CDC's Emerging Infection Program, 2000.
5. 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.
6. Jay JM, Modern Food Microbiology, 6th edition. Aspen Publishers, Maryland, 2006.
7. 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.
8. 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.
9. Okonko, Iheanyi Omezuruike, Ogunjobi, Adeniyi Adewale, Fajobi, Enobong Aloysius. Comparative studies and microbial risk assessment of different Ready-to-Eat RTE frozen sea-foods processed in Ijora-olopa, Lagos State, Nigeria, 2008.
10. 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.
11. 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 DC, Area. Applied Environ. Microbiol., 2001; 67:5431-5436.