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## Examination of microbiological quality of boondi raita served at selected restaurants

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### Abstract

**Introduction:** It has been a challenge for the restaurateurs at a global level to maintain food quality. Food quality not only deals with organoleptic properties but also with the nutritional adequacy and microbiological safety of the food served. However, a number of food poisoning outbreaks exhibit the reality of food served at restaurants.

**Objectives:** The research was carried out with the following objectives:

- To analyse the microbiological adequacy of boondi raita served at the selected restaurants
- To identify the pathogens present in boondi raita served at the studied restaurants

**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 recipe prepared was also analyzed for comparative analysis.

**Results:** Boondi raita served at public restaurants had the highest number of bacterial colonies with a mean value of  $1.30 \times 10^{11}$  CFU per ml. at  $10^{-7}$  dilution. The boondi raita prepared as standardized recipe had shown minimum CFUs at all the dilutions. There was presence of *Bacillus cereus*, *E. coli*, *Pseudomonas*, *Aspergillus* and *Penicillium* in boondi raita of private eateries whereas *Bacillus cereus*, *Aspergillus* and *Penicillium* were detected in public sector samples. Boondi raita of fast food restaurants also had *Bacillus cereus*, *Aspergillus* and *Penicillium* in unsafe amounts.

**Conclusion:** Although results of t-test showed a significant difference in bacterial as well as fungal CFUs among all three types of selected restaurants yet all were having pathogens in unsafe limits. The t-test outcomes however highlighted fungal contamination to be less in case of private and fast food restaurants as compared to public restaurants.

**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.

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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 analyse the microbiological adequacy of boondi raita served at the selected restaurants
- To identify the pathogens present in boondi raita 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) [2]. These pathogens, though, usually cause self-limiting gastroenteritis, complications may occur, resulting in more severity. *Staphylococcus aureus* is one of the most common agents in bacterial food poisoning outbreaks (Adwan *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 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. Thus, assessment of the chemical quality of these food products is very important to improve health of consumers (Jay, 2006) [5]. 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 [6] 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) [9].

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 aureus* (5.9 per cent) (Okonko *et al.*, 2008) [8]. In Byrne *et al.*, 2008 [3] in his studies showed similar results in a meat industry. Report in journal indicates that the highest total viable count was observed at 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) [3].

A majority of food poisoning outbreaks is associated with improper holding that occurs in institutional settings (CDC, 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.

*Bacillus cereus*, an infectious cause of foodborne illness, accounted for 2% of outbreaks with confirmed etiology that were reported to CDC during 1973-1987. On July 21, 1993, the Lord Fairfax (Virginia) Health District received reports of acute gastrointestinal illness that occurred among children and staff at two jointly owned child day care centers following a catered lunch. This report summarizes the investigation of this outbreak.

### 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:** Serial Dilutions of Boondi Raita



**Photo 2:** Studying Colony Morphology

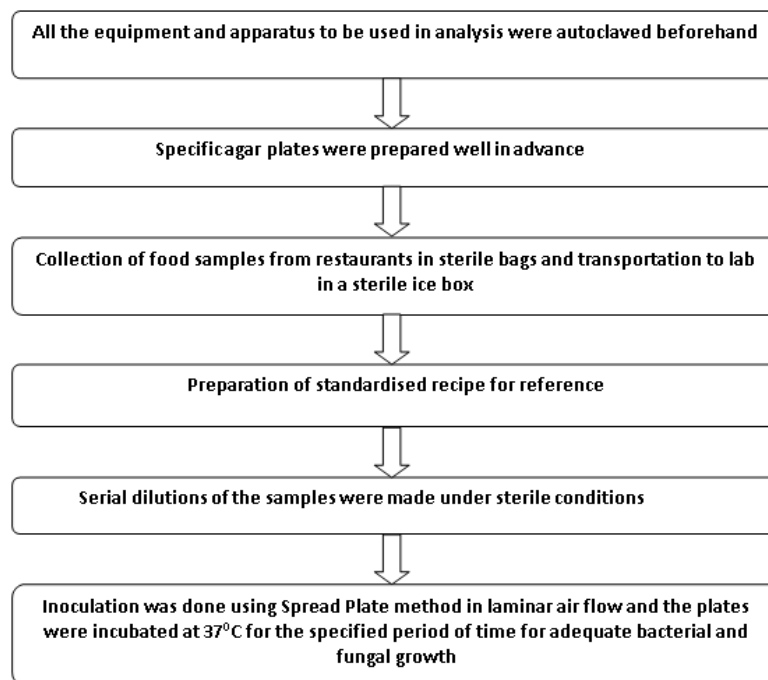
### Method

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 boondi raita 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 Potato Dextrose Agar (PDA) for fungal colonies at specified serial dilutions ( $10^{-6}$  to  $10^{-8}$  for bacterial growth and  $10^{-4}$  to  $10^{-6}$  for fungal growth) under sterile conditions in

laminar air flow. This was thereafter followed by a controlled incubation at  $37^{\circ}\text{C}$  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 Boondi Raita 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). Boondi raita 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 boondi raita served at private, public and fast food restaurants.

### Microbiological Examination of Boondi Raita

The samples of boondi raita 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 and discussed thereafter.

**Table 1:** Comparison of Microbial Flora (CFU/ml) of Boondi Raita served at Private, Public and Fast Food Restaurants

BR	Dilution	Mean Bacterial CFU**	Bacterial CFU**/ml	Dilution	Mean Fungal CFU**	Fungal CFU**/ml
R1	10 <sup>-6</sup>	195	1.95 × 10 <sup>9</sup>	10 <sup>-4</sup>	2	2.00 × 10 <sup>5</sup>
	10 <sup>-7</sup>	88	8.8 × 10 <sup>9</sup>	10 <sup>-5</sup>	1	1.00 × 10 <sup>6</sup>
	10 <sup>-8</sup>	49	4.9 × 10 <sup>10</sup>	10 <sup>-6</sup>	0	0
R2	10 <sup>-6</sup>	294	2.94 × 10 <sup>9</sup>	10 <sup>-4</sup>	53	5.30 × 10 <sup>6</sup>
	10 <sup>-7</sup>	130	1.30 × 10 <sup>11</sup>	10 <sup>-5</sup>	44	4.40 × 10 <sup>7</sup>
	10 <sup>-8</sup>	35	3.5 × 10 <sup>10</sup>	10 <sup>-6</sup>	1	1.00 × 10 <sup>7</sup>
R3	10 <sup>-6</sup>	248	2.48 × 10 <sup>9</sup>	10 <sup>-4</sup>	2	2.00 × 10 <sup>5</sup>
	10 <sup>-7</sup>	165	3.65 × 10 <sup>10</sup>	10 <sup>-5</sup>	1	1.00 × 10 <sup>6</sup>
	10 <sup>-8</sup>	50	5.00 × 10 <sup>10</sup>	10 <sup>-6</sup>	1	1.00 × 10 <sup>7</sup>
SR*	10 <sup>-6</sup>	39	1.50 × 10 <sup>8</sup>	10 <sup>-4</sup>	0	0
	10 <sup>-7</sup>	26	2.60 × 10 <sup>9</sup>	10 <sup>-5</sup>	0	0
	10 <sup>-8</sup>	15	3.90 × 10 <sup>10</sup>	10 <sup>-6</sup>	0	0

\*Standardised Recipe

\*\* Colony Forming Units

The mean scores for bacterial colonies in boondi raita of the private restaurants are 195, 88 and 49 respectively at 10<sup>-6</sup>, 10<sup>-7</sup> and 10<sup>-8</sup> dilution. The same were found to be 294, 130 and 35 in case of public sector units while 248, 165 and 50 in samples of fast food eateries. It was noted that the standardized recipe for boondi raita however recorded 39, 26 and 15 mean bacterial colonies at the same specified dilutions. While appraising the results for fungal colonies, it was noticed that the mean scores for fungal colonies of the samples of boondi raita from private restaurants are 2, 1 and 0 respectively at 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> dilution. Public restaurants have surpassed the level for fungal colonies at the same dilution with mean values of 53, 44 and 1 respectively.

However, the fast food restaurants are at par with the private counterparts with mean fungal colonies score of 2, 1 and 1 respectively at 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> dilution. No fungal growth was noticed in the standardized recipe at any dilution. It was observed from Table 2 that boondi raita served at public restaurants had the highest number of bacterial colonies with a mean value of 1.30 × 10<sup>11</sup> CFU per ml. at 10<sup>-7</sup> dilution. However, not much difference was found in the bacterial CFUs/ml. in the boondi raitas of private and fast food restaurants at all the specified dilutions. The boondi raita prepared as standardized recipe had shown minimum CFUs at all the dilutions.

**Table 2:** Comparison of Bacterial CFUs in Boondi Raita 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	- 4.372	16	.000**
	Equal variances not assumed		- 4.372	13.853	.001*
CFU	Equal variances assumed	Private versus Fast Food	.608	16	.552
	Equal variances not assumed		.608	14.167	.553
CFU	Equal variances assumed	Public versus Fast Food	5.214	16	.000**
	Equal variances not assumed		5.214	11.146	.000**

\* Significant at p ≤ 0.05

\*\*Significant at p ≤ 0.001

Table 2 depicts the results of t-test carried out on private versus public restaurants, private versus fast food restaurants and public versus fast food restaurants. It was observed that a significantly high difference was found between bacterial colonies in boondi raita of private versus public restaurants as well as public versus fast food restaurants owing to a p-value of 0.000 at 99 per cent confidence level. The difference was however found to be insignificant in case of private versus fast food restaurants with a p-value of 0.552 at the same confidence level. This means that boondi raita served at private as well as fast food establishments is similar in microbiological quality.

Plates 1 to 9 represent the agar plates of bacterial colonies on various samples of boondi raita procured from the selected restaurants. These CFUs were counted from these plates and the bacteria were then isolated on broth tubes for further examination.

**Plate 1:** Bacterial Colonies on NA Plate of Boondi Raita of Private Restaurants (10<sup>-6</sup> Dilution)



**Plate 2:** Bacterial Colonies on NA Plate of Boondi Raita of Private Restaurants ( $10^{-7}$  Dilution)



**Plate 6:** Bacterial Colonies on NA Plate of Boondi Raita served at Public Restaurants ( $10^{-8}$  Dilution)



**Plate 3:** Bacterial Colonies on NA Plate of Boondi Raita of Private Restaurants ( $10^{-8}$  Dilution)



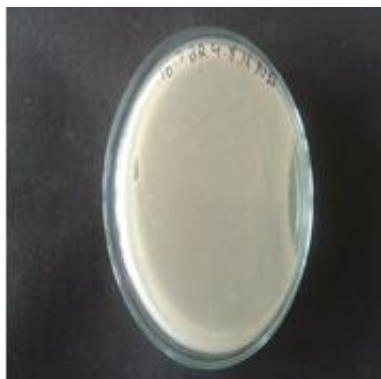
**Plate 7:** Bacterial Colonies on NA Plate of Boondi Raita served at Fast Food Restaurants ( $10^{-6}$  Dilution)



**Plate 4:** Bacterial Colonies on NA Plate of Boondi Raita served at Public Restaurants ( $10^{-6}$  Dilution)



**Plate 8:** Bacterial Colonies on NA Plate of Boondi Raita served at Fast Food Restaurants ( $10^{-7}$  Dilution)



**Plate 5:** Bacterial Colonies on NA Plate of Boondi Raita served at Public Restaurants ( $10^{-7}$  Dilution)



**Plate 9:** Bacterial Colonies on NA Plate of Boondi Raita served at Fast Food Restaurants ( $10^{-8}$  Dilution)

Table 3 elicits the results of t-test performed on private versus public restaurants, public versus fast food restaurants and private versus fast food restaurants. A significant difference was found between fungal colonies in boondi raita of private versus public restaurants and public versus fast food restaurants with p-value of 0.001 at 95 per cent confidence

level in both the comparisons whereas the p-value of 0.332 at the same level of confidence shows insignificant difference in fungal CFUs between private and fast food restaurants. These outcomes highlight that the fungal contamination is less in case of private and fast food restaurants while the food served at public restaurants is of more deteriorated quality.

**Table 3:** Comparison of Fungal CFUs in Boondi Raita 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	-3.945	16	.001*
	Equal variances not assumed		-3.945	8.021	.004*
CFU	Equal variances assumed	Private versus Fast Food	-1.000	16	.332
	Equal variances not assumed		-1.000	12.800	.336
CFU	Equal variances assumed	Public versus Fast Food	3.905	16	.001*
	Equal variances not assumed		3.905	8.007	.005*

\* Significant at  $p \leq 0.05$

Plates 10 to 18 represent the agar plates of fungal colonies on various samples of boondi raita 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.



**Plate 10:** Fungal Colonies on PDA Plate of Boondi Raita served at Private Restaurants ( $10^{-4}$  Dilution)



**Plate 11:** Fungal Colonies on PDA Plate of Boondi Raita served at Private Restaurants ( $10^{-5}$  Dilution)



**Plate 12:** Fungal Colonies on PDA Plate of Boondi Raita served at Private Restaurants ( $10^{-6}$  Dilution)



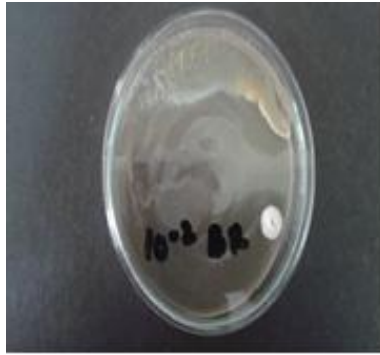
**Plate 13:** Fungal Colonies on PDA Plate of Boondi Raita served at Public Restaurants ( $10^{-4}$  Dilution)



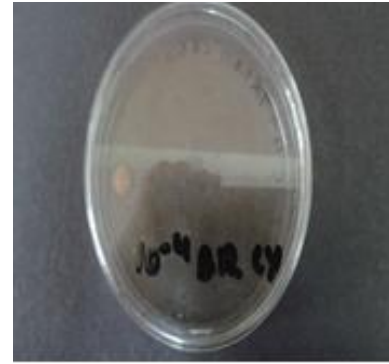
**Plate 14:** Fungal Colonies on PDA Plate of Boondi Raita served at Public Restaurants ( $10^{-5}$  Dilution)



**Plate 15:** Fungal Colonies on PDA Plate of Boondi Raita served at Public Restaurants ( $10^{-6}$  Dilution)



**Plate 16:** Fungal Colonies on PDA Plate of Boondi Raita served at Fast Food Restaurants ( $10^{-4}$  Dilution)



**Plate 18:** Fungal Colonies on PDA Plate of Boondi Raita served at Fast Food Restaurants ( $10^{-5}$  Dilution)



**Plate 17:** Fungal Colonies on PDA Plate of Boondi Raita served at Fast Food Restaurants ( $10^{-5}$  Dilution)

**Morphological examination of the microbial colonies**

Tables 4 to 6 depict 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°C 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 Bacterial Colonies in Boondi Raita served at Private Restaurants

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

**Table 5:** Morphological Examination of the Bacterial Colonies in Boondi Raita served at Public Restaurants

Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
BR	$10^{-6}$	1	Circular Irregular	Entire Undulate	Raised Raised	Punctiform Large	Smooth Rough	Shiny Cloudy	Yellow White	Opaque Translucent
		2	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Yellow	Opaque
		3	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Yellow	Opaque
	$10^{-7}$	1	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Yellow	Opaque
		2	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Yellow	Opaque
		3	Circular	Entire	Raised	Punctiform	Smooth	Shiny	Yellow	Opaque
	$10^{-8}$	1	Circular Irregular	Entire Undulate	Raised Raised	Punctiform Large	Smooth Rough	Shiny Cloudy	Crème White	Opaque Translucent
		2	Irregular	Undulate	Raised	Large	Rough	Cloudy	White	Translucent
		3	Irregular	Undulate	Raised	Large	Rough	Cloudy	White	Translucent

**Table 6:** Morphological Examination of the Bacterial Colonies in Boondi Raita served at Fast Food Restaurants

Food Sample	Dilution	Plate	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical Property
BR	$10^{-6}$	1	Circular Irregular Rhizoid	Entire Undulate Rhizoid	Flat Flat Raised	Punctiform Medium Large	Smooth Rough Smooth	Shiny Dull Cloudy	Crème Buff White	Translucent Opaque Opaque
		2	Circular Irregular	Entire Undulate	Flat Flat	Punctiform Medium	Smooth Rough	Shiny Dull	Crème Buff	Translucent Opaque
		3	Circular Irregular	Entire Undulate	Flat Flat	Punctiform Medium	Smooth Rough	Shiny Dull	Crème Buff	Translucent Opaque

			Rhizoid	Rhizoid	Raised	Large	Smooth	Cloudy	White	Opaque
	10 <sup>-7</sup>	1	Circular Irregular	Entire Undulate	Flat Flat	Punctiform Medium	Smooth Rough	Shiny Dull	Crème Buff	Translucent Opaque
		2	Circular Irregular	Entire Undulate	Flat Flat	Punctiform Medium	Smooth Rough	Shiny Dull	Crème Buff	Translucent Opaque
		3	Circular Irregular	Entire Undulate	Flat Flat	Punctiform Medium	Smooth Rough	Shiny Dull	Crème Buff	Translucent Opaque
	10 <sup>-8</sup>	1	Circular Irregular Rhizoid	Entire Undulate Rhizoid	Flat Flat Raised	Punctiform Medium Large	Smooth Rough Smooth	Shiny Dull Cloudy	Crème Buff White	Translucent Opaque Opaque
		2	Circular Irregular Rhizoid	Entire Undulate Rhizoid	Flat Flat Raised	Punctiform Medium Large	Smooth Rough Smooth	Shiny Dull Cloudy	Crème Buff White	Translucent Opaque Opaque
		3	Circular Irregular Rhizoid	Entire Undulate Rhizoid	Flat Flat Raised	Punctiform Medium Large	Smooth Rough Smooth	Shiny Dull Cloudy	Crème Buff White	Translucent Opaque Opaque

**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.

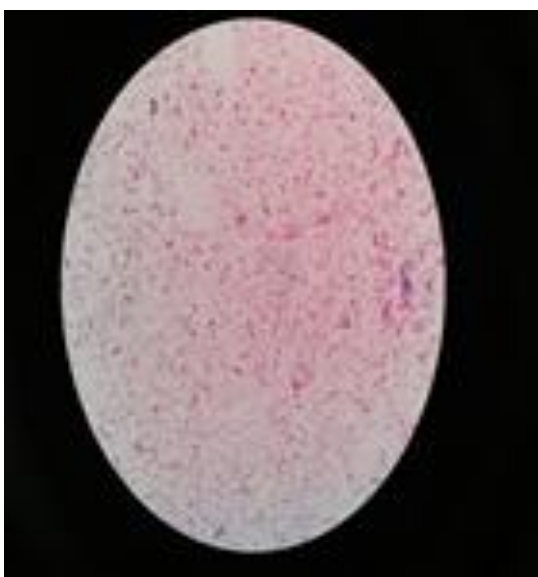
**Gram staining of bacterial smears**

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**

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**



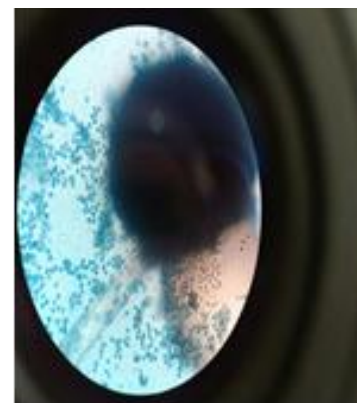
**Plate 19:** A mixed gram stain of *E. coli* (gram-negative rods, in red) and *B. cereus* (gram-positive rods, in purple) in smear of Boondi Raita served at Private Restaurants



**Plate 20:** A mixed gram stain of *B. subtilis* (gram-positive rods with terminal endospores present in long chains, in purple) and *B. cereus* (gram-positive rods present singly, in purple) in smear of Boondi Raita served at Public Restaurants



**Plate 21:** A gram stain of *B. cereus* (gram-positive rods present singly, in purple) in smear of Boondi Raita served at Fast Food Restaurants

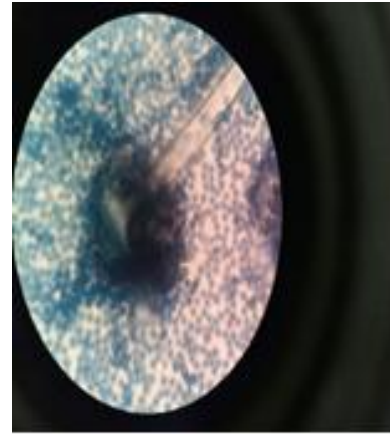


**Plate 22:** *Penicillium* in smear of Boondi Raita served at Private Restaurants





**Plate 23:** *Aspergillus* in smear of Boondi Raita served at Public Restaurants



**Plate 24:** *Penicillium* in smear of Boondi Raita served at Fast Food Restaurants

### a) 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 Table 7.

**Table 7:** Microbes identified in Boondi Raita served at Selected Restaurants.

S. No.	Food Sample	Type of Restaurant	Microbes isolated from Samples
1	Boondi Raita	Private	<i>Bacillus cereus, E.coli, Pseudomonas, Aspergillus, Penicillium</i>
2	Boondi Raita	Public	<i>Bacillus cereus, Bacillus cereus, Aspergillus, Penicillium</i>
3	Boondi Raita	Fast Food	<i>Bacillus cereus, Aspergillus, Penicillium</i>

### Conclusion

Boondi raita served at public restaurants had the highest number of bacterial colonies with a mean value of  $1.30 \times 10^{11}$  CFU per ml. at  $10^{-7}$  dilution. The boondi raita prepared as standardized recipe had shown minimum CFUs at all the dilutions. The total viable counts however were analysed to be higher than the acceptable levels in all three types of studied restaurants. There was presence of *Bacillus cereus, E.coli, Pseudomonas, Aspergillus, Penicillium* in boondi raita of private eateries whereas *Bacillus cereus, Aspergillus, Penicillium* were detected in public sector samples. Boondi raita of fast food restaurants had *Bacillus cereus, Aspergillus, Penicillium* in unsafe amounts (Table 7). Although results of t-test showed a significant difference in bacterial as well as fungal CFUs among all three types of selected restaurants yet all were having pathogens in unsafe levels. The t-test outcomes however highlighted fungal contamination to be less in case of private and fast food restaurants in comparison to public restaurants.

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