Microbiological survey of ready-to-eat foods and associated preparation surfaces in cafeterias of public sector universities

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ABSTRACT
A microbiological and sanitation survey of ready-to-eat (RTE) food samples, cutlery, utensils, and hands of food handlers, food preparation surfaces, serving counters, washing areas, and refrigerator handles were conducted. The samples were collected using environmental swabs, and these samples were analyzed using standard plating techniques. Samples were characterized after aerobic plate counts (APC) and isolated for mesophilic aerobic bacteria (MAB), total coliforms, Staphylococcus aureus, Escherichia coli, Salmonella spp., Shigella spp., Pseudomonas spp., Enterobacter spp., Klebsiella spp., and yeast. This study suggested that good-hygiene practices can minimize bacterial counts, thereby decreasing the reservoirs for bacterial contamination. It also improves the hygienic condition of RTE-foods in the cafeteria of Universities.

1. Introduction
The Food and Agricultural Organization reported that approximately 1.3 billion tons/yr of food waste are generated globally [1–3], therefore signifying one-third of the global annual food generation [4]. The changing world trends and recent economic and food crisis alarmed policymakers, government officials, and common people to think again critically on depleting sources of harvesting and food security. Many developing countries are experiencing perpetual food shortages; around 925 million people are hungry because of extreme poverty and are often victimized by malnutrition, starvation, and stunted growth. Approximately 2 billion people are consuming insecure food. Pakistan is ranked at 59 by the International Food Policy Institute with a 27.5% prevalence of underweight children below five years; this figure is targeted below 20 in Millennium Development Goals for 2015, 26% of the undernourished population, 20.7% Global Hunger Index, and 8.7% mortality rate under the age of 5 [5].

The recent global shift towards consumption of ready-to-eat (RTE) foods has increased exponentially due to its prompt availability and nutritious meals [6]. On the contrary, the safety and microbiological quality of that food have always been questioned, especially when considering local retailers [7–10]. Microbial load on raw material is always an important factor that increases with transportation, handling, processing, and displaying RTE foods [11–14]. RTE foods like Burger, Chaat, chatni (sauce), salad, and sandwiches are prepared on-site with bare hands, which provide transmission of hand microflora and associated food-borne pathogens to enter the food chain and may cause food intoxication or food poisoning. These incidences are reported throughout the world [15–20].

RTE foods are microbiologically investigated and examined during preparation in health care settings, schools, shopping centers, restaurants, factories, retail outlets, and domestic kitchens worldwide [21–27]. Several outbreaks of food intoxication and food poisoning are reported in Schools, Colleges, universities, and Hospitals of Pakistan and worldwide [28–30].

This study aimed to evaluate the microbiological quality of ready-to-eat foods served to the students, employees, and faculty members in the cafeteria of public sector universities in Pakistan. The study’s main focus was to examine RTE foods for Aerobic Colony Count (ACC or CC), E.coli count (EC), and identification of Escherichia coli, Klebsiellae, Shigella, Salmonella, Streptococcus and Staphylococcus spp., Pseudomonas spp. and yeasts.
2. Material and methods

2.1 Description of RTE foods

Ready to eat foods are mostly prepared in the cafeterias and sold among the University students, Employees, and Faculty daily. The raw material is brought from the vicinity, whereas some RTE foods are also brought from outside, where they are displayed for sale. This study was conducted to evaluate RTE food available in a public sector university cafeteria during the spring session. Seven canteens were surveyed twice, and later on, random samples were also assessed to minimize the chances of error. The samples of RTE foods; Biryani, burger, cake, ketchup, chaat (Sauce), patties, samosa, roll, salad, salan (curry), and swabs of associated utensils like preparation knives, spoons, plates, glasses, cutting boards, preparation surfaces, cleaning cloths, serving counter, washing area, refrigerator handle and hands of food handlers. A total of twenty-three (23) different categories were selected, and samples were collected at different intervals of time (Table 1). Out of these RTE food samples: the highest number of samples was the Biryani, followed by the Burger. Samosa samples were analyzed in two portions: the outer part named as samosa (B) and the inner part containing the potato mash material with added other ingredients named as samosa (A). Table 1 also shows the number of samples collected for associated preparation surfaces (APS). A total of eleven (11) APS, including food serving utensils and hands of food handlers, were analyzed; the highest numbers of samples were taken from food handlers’ hands.

2.2 Sample collection

After acquiring the cafeteria owners’ consent, RTE foods were purchased, and a portion of each required sample was aseptically transferred to sterile containers to minimize the risk of cross-contamination. Similarly, commercially available sterile swabs (Oxford, UK) were used for sampling of associated preparation surfaces, utensils, and hands of food handlers by moistening the swab with synthetic transport medium containing meat extract 1 g per l, riboflavin 0.2 g l⁻¹, biotin 0.2 g l⁻¹, asparagine 0.1 g l⁻¹, peptone 1 g l⁻¹, glucose 1 g l⁻¹ of distilled water prepared in sterile test tubes containing 5 mL of this medium. The medium’s pH was adjusted to 7.2 using a portable pH meter, internally calibrated using pH buffer solution of 4, 7, and 9. The pH of solutions was adjusted using acidic and basic solutions considering the cross-reactivity and binding affinities of the anions [31]. Samples were aseptically collected by placing the tip on the surface of the sampling portion and slightly rotating the swab between the thumb and point the finger in to and fro direction at a right angle to each other for 15 seconds [32]. Later, all samples were transported to the laboratory and were analyzed on the same day of collection.

2.3 Sample processing and analysis

For RTE foods processing, 20 g of each sample was weighed and dissolved in 180 mL of a sterile peptone-saline solution containing 0.1% of peptone and 0.85% NaCl. Samples were homogenized after vigorous shaking for 30–45 seconds. Whereas, for the assessment of utensils, associated preparation surfaces, and hands of food handlers; each swab sample was aseptically shifted from transport medium to 10 mL tube of sterile nutrient broth (Merck, Germany) same homogenization procedure was repeated [13,21,33].

After mixing, the serial dilutions were prepared for all RTE food samples in sterile peptone saline solution, which were then cultured on agar plates duplicated through the standard spread plate and pour plate method [32]. These cultured agar plates were then incubated aerobically at 37°C ± 2°C for 24 hours. Whereas for fungi isolation Sabraud Dextrose Agar SDA (Scharlau, Spain) plates were kept at room temperature (25°C) for one week. Colony-forming units (CFU) were counted through the colony counter (Suntex, Taiwan) and calculated per mL for each RTE food sample and per cm² for associated preparation surfaces, including the hands of food handlers. RTE foods and swabs of associated preparation surfaces, utensils, and hands of food handlers were analyzed for aerobic bacteria, including Enterobacteriaceae family, Staphylococcus aureus, and Streptococcus spp.

3. Results

The emphasis of this study was to evaluate the level of microbial contamination in: (a) Food samples of ready-to-eat available in the cafeteria of a public sector university, (b) Associated preparation surfaces including cutlery, utensils, and (c) Hygiene of food handlers with respect to their hands. A total of 445 RTE food samples of 11 categories and 505 samples of associated preparation surfaces, including, cutlery, utensils, and the
hands of food handlers, have been analyzed for aerobic colony count and later for further isolation and identification.

3.1 CFU

Samples of RTE, APS, and hand hygiene were separately processed through dilution in the heterotrophic plate count (HPC). The results for aerobic plate count, APC of all the representative samples was counted, and later, an average log of CFU per mL (mL⁻¹) or CFU per gram (g⁻¹) or CFU per square cm (cm⁻²) was calculated (Figure 1). The Salad samples, Chaat, and Burger counted Log CFU/g as 8.1, 7.49, and 7.324 indicating the samples consume quality of tap water along with exposure to ambient air environment. Likewise, samples from the APS demonstrated the highest aerobic plate count (APC) in Log CFU/g for the sample of the Knife, Cleaning cloth, and washing area ranging from 7.85, 8.2, and 7.52, respectively. The samples collected from hand were counted for APC as 7.9 logCFU/g indicating hand hygiene played a significant role in the overall preparation and serving of cafeterias’ food items. Furthermore, tap water in the washing of vegetables and preparation of salad, burgers, and other food items impact the microbial community.

3.2 Microorganisms isolated from ready-to-eat foods

3.2.1 Gram-positive species (GP)

RTE foods of a total of 11 commonly used items were focused in this study, among them 11.11% samples cultured positive for Biryani, with maximum isolates of non-pathogenic species (57.14% of Staphylococcus saprophyticus). Burger items showed microbial growth in 46.67% of samples; among these, 28.57% of GP species were isolated and identified as Staphylococcus aureus. Sauce demonstrated growth in 57.78% samples, among them 61.54% identified and isolated as GP (Staphylococcus aureus 15.38%, β-hemolytic streptococci 3.85%); 37 samples of Chaat indicated growth in 64.86% with 25% GP species, and all of them were isolated as non-pathogenic Staphylococcus saprophyticus. Similarly, 18.18% samples

![Figure 1.](image-url) Aerobic plate count of monitored samples. Log of CFU/g and Log of CFU/cm² of (A) Ready-to-eat samples and (B) Associated preparation surfaces represented in black bars, coliform count (light shadowed bars), E.coli (stripped bars), and Gram positive Staphylococcus spp. also represented as SAC (black dotted lines).
of patties were cultured as positive for microbial contamination, which identified 25% Gram-positive species including 10% *Pseudomonas* spp., in Samosa part (A), among all positive samples, 60% were identified as GP and isolated as (*Staphylococcus aureus* 10%, *Streptococcus* spp. 20%, and non-pathogens 30%). In contrast, for Samosa part (B), only two samples showed growth with no GP pathogen. Salan samples were cultured 30%, GP, which were isolated (*Streptococcus* spp. 30%, and *Staphylococcus saprophyticus* 10%), whereas 38.10% of all roll samples were cultured positive, among them 62.5% were GP with no pathogenic species (Figure 2).

### 3.2.2 Gram-negative species (GN)
As discussed earlier in Biryani samples, 42.86% were isolated Gram-negative with representative species *Escherichia coli*. 64.29% of the burger samples were identified as GN and isolated (*E. coli* 39.29%, *Salmonella* spp. 10.71%, and *Shigella* spp. 14.29%), and the yeasts was isolated as 7.14%. All cake samples showed no growth of GN species however, 100% yeast were isolated. Sauce contained 34.62% *E.coli*, Chaat samples determined (*E.coli* 58.33% and *Enterobacter* spp 16.67%). Patties showed growth of yeast up to 30%. *E.coli* was isolated from samples of Samosa part (a) and (b) as 40% and 50% respectively. Whereas *E.coli*, *Shigella* spp., *Enterobacter* spp. and yeast were isolated from salad samples as 37.50%, 8.33%, 8.33%, and 4.17% respectively; Samal samples indicated microbial growth of *E.coli* and yeast as 50%, 20%, and roll samples depicted 37.50% of yeast (Table 2).

#### 3.3 Microorganisms isolated from associated preparation surfaces
In this category, 10 parameters were selected, Aerobic plate counts APC for each of them was calculated. Later on, they were further identified for coliform bacteria, including *E.coli*, *Staphylococcus* spp. and *Streptococcus* spp. All preparation knife samples were

Table 2. Results of gram staining.

| Sample Type          | Total positive samples | Gram-negative population | Gram-positive population | Yeast |
|----------------------|------------------------|---------------------------|--------------------------|-------|
| Biryani              | 11.11                  | 42.86                     | 57.14                    | 0     |
| Burger               | 46.67                  | 64.29                     | 28.57                    | 7.14  |
| Cake                 | 8.82                   | 0                         | 0                        | 100   |
| Ketchup              | 57.78                  | 34.62                     | 61.54                    | 3.85  |
| Sauce                | 68.86                  | 75                        | 25                       | 0     |
| Paties               | 18.18                  | 25                        | 0                        | 75    |
| samosa a             | 33.33                  | 40                        | 60                       | 0     |
| samosa b             | 6.67                   | 50                        | 0                        | 0     |
| Salad                | 68.57                  | 54.17                     | 41.67                    | 4.17  |
| Salan                | 43.48                  | 50                        | 30                       | 20    |
| Roll                 | 38.10                  | 62.50                     | 37.50                    |       |
| preparation knives   | 100                    | 65                        | 35.71                    | 0     |
| Spoon                | 47.62                  | 50                        | 50                       | 0     |
| Plate                | 52.38                  | 27.27                     | 45.45                    | 27.27 |
| Glass                | 40.48                  | 23.53                     | 76.47                    | 0     |
| cutting board        | 66.67                  | 80                        | 0                        | 0     |
| preparation area     | 62.68                  | 59.09                     | 4.55                     | 36.36 |
| refrigerator handle  | 100                    | 47.5                      | 37.5                     | 15    |
| serving counter      | 84.44                  | 31.58                     | 57.89                    | 10.53 |
| cleaning cloth       | 86.27                  | 36.36                     | 50                       | 13.64 |
| washing area         | 74.29                  | 50                        | 7.69                     | 42.31 |
| Hands of food handlers| 95.15                  | 34.4                      | 59.2                     | 6.4   |

* All positive samples were further processed for gram reaction and later, samples were identified for Enterobacteriaceae, *Staphylococci* spp. and *Streptococci* spp.

Figure 2. % distribution of Gram positive (G+ ve), Gram negative (G-ve), and Yeast isolated from the samples of (A) Ready-to-eat (RTE) sample types, and (B) Associated preparation surfaces (APS) sample types including hands of food handlers.
found positive with APC range 7 log of CFUcm⁻². Among these 35.71% were identified as GP with a maximum of *Pseudomonas* spp, 45% and *α-haemolytic Streptococci* 5%.

Spoon samples showed 47.62% of microbial growth with no GP pathogen. Among all plate samples, 45.45% were identified as GP with 4.55% *α-haemolytic Streptococci; similarly for glass, preparation area, refrigerator and serving counter samples 11.76%, 27.27%, 27.50%, 23.68% of *Pseudomonas* spp and 5.88%, 4.55%, 15%, 10.53% *α-haemolytic Streptococci* were isolated. In refrigerator handle, 2.5% *Staphylococcus aureus* was isolated, and in cleaning cloth, 11.36% *Staphylococcus aureus* and 13.64% *α-haemolytic Streptococci* were isolated, whereas, in the washing area, 7.69% *α-haemolytic Streptococci* were identified (Figure 3).

All associated preparation surfaces, including cutlery and utensils, were analyzed for Gram-negative (GN) species. The main emphasis of the study was to evaluate microbial status for analysing the chances of food-borne infections. Therefore *Enterobacteriaceae* was focused upon. In the knives samples used for preparation, 35% isolates were identified as *Escherichia coli*, 10% *Klebsiella* spp., 5% *Shigella* spp. 10% *Morganella* spp.; in all spoon samples categorized as GN had 40% *E.coli* and 10% *Klebsiella* spp; samples of the plate had 22.73% isolates of *E.coli* and 4.55% *Shigella* spp. In glass samples, cutting board, refrigerator handle, preparation area, and serving counter was 11.76%, 30%, 20%, 22.73%, and 5.26% *E.coli* was isolated. Similarly, 9.09%, 2.63% *Klebsiella* spp. were isolated from preparation area and serving counter, respectively. Meanwhile, from cleaning cloth 27.27% *E. coli*, 4.5% *Shigella* spp., and 4.5% *Enterobacter* spp. was isolated and at washing area 11.54% *E. coli*, 3.85% *Shigella* spp. were isolated. In addition, isolates of *Pseudomonas* spp. were identified from the samples of knife, glass, cutting board, preparation areas, refrigerator handle, serving counter, and washing area with an accumulative percentile of 5%, 11.76%, 50%,

![Figure 3](image-url)

*Figure 3.* % distribution of bacterial species and fungi isolated from the samples of (A) Ready-to-eat (RTE) sample types, and (B) Associated preparation surfaces (APS) sample types including hands of food handlers.
27.27%, 27.50%, 23.68%, and 34.62%. A relatively high prevalence of GN isolates indicated contamination primality from preparation materials and water used for washing. Since the knife was infrequently used, it was observed, cutting material stuck its edges and might be a transfer source. The water quality of the tap was separately assessed and prepared separately; the results have deliberately not been included in this manuscript.

Among all plate samples, 52.38% showed growth with microbial colonies ranging 7.672 log of CFU mL$^{-1}$, glass samples were positive for 40.48% with CFU log of 7.74 mL$^{-1}$, the cutting board showed growth for 66.67% with 7.5 log of CFU mL$^{-1}$, whereas all samples of cleaning cloth, preparation area, refrigerator handle, and washing area showed heavy growth on nutrient agar media (Merck, Germany) with Aerobic plate count of 8.45, 7.69, 7.63, and 8.12 log of CFU mL$^{-1}$ respectively (Figure 1).

### 3.4 Microorganisms isolated from hands of food handlers

96.15% of all samples taken from the hands of food handlers showed growth of microorganisms with 14.4% *Escherichia coli*, 3.2% *Shigella* spp., 7.2% *Klebsiella* spp., 9.6% *Pseudomonas* spp., 4% β-*haemolytic Streptococci*, 17.6% *Staphylococcus aureus*, and 6.4% yeast.

### 4. Discussion

During preparation and serving of foods in the cafeteria, hygienic conditions must strictly be followed [34,35]; otherwise, chances of food-borne disease increase to consumers [36,37].

Biryani is a rice-based food made with spices, rice, meat, fish, eggs, or vegetables. This dish is popular in all Asian. Biryani is taken very often at lunchtime by 90% of people working in every environment; therefore, special precautionary measures must be obtained for providing maximum food safety and security. While survey, we found 11% samples positive for microbial growth having log of CFU 6.4, 4.8, and 3.1 per gram of APC, CC, and EC. Usually, biryani is kept in a closed container to keep warm but may be due to frequent opening, air microbes, and the use of bare unwashed hands might contaminate it. Samples of burger showed 7.3 log CFU/g, which indicate microbial accumulation from unwashed hands, preparation surfaces, utensils, salads, vegetables, tap water, and open containers. All samples of ketchup were found sterile, probably due to its packing. Comparing to results of salad vegetable samples reported by [38], our results differed invarably as 68% showed microbial accumulation with 8.1, 5, 4, and 1.2 log CFUg$^{-1}$ of APC, CC, EC, and SAC, respectively. Variability in results was characterized by associated preparation surfaces, including utensils and hands of food handlers and tap water quality.

Comparative results of preparation knives for APC, CC, and EC were double than previously reported by [13]. During the survey, it was found in most of the cafeterias that preparation knives were exchanged among food handlers for preliminary processing of RTE foods. It seemed as these knives were in use for a long period without maintenance and proper cleaning, which increases chances of microbial accumulation and easy transfer among workers and in the food.

Results for spoon samples were almost the same as previously reported by [13], with variation in coliform count from 2.2 to 5.6 log CFU cm$^{-2}$. Other utensils as plate and glass showed high APC as 5 and 3 log CFU cm$^{-2}$. After these results, cafeterias were surveyed again to know the cause of such increased contamination. In response to the question related to the washing of utensils, cafeterias owners responded well, which indicated their cleaning concern. However, at two sampling points, haphazard washing and improper placement of washed plates, spoons, and glass were observed. Samples of tap water were also taken for analysis to know the status of water used for washing and other purposes. These water samples were analyzed in the Laboratory of water quality in the Department of Environmental Engineering NED University of Engineering and Technology. Results showed a high number of total and faecal coliforms. Relevant authorities were informed of these findings. Water disinfection dosage was suggested for implementation in the main underground water tank to achieve desired water quality levels in the overall University.

Cutting boards were found less often to be utilized to prepare food, some of them in poor condition; some were having cracks, which may enhance microbial accumulation and formation of biofilms. 4.2 log CFU cm$^{-2}$ APC was calculated, which was a little lower than previously reported by [13].

The refrigerator handle showed log CFU cm$^{-2}$ for APC, CC, EC, and SAC as 6.7, 3.4, and 3.2, respectively. It was noticed that the refrigerator was frequently used by cafeteria staff and consumers for taking of their required items (often soft drinks, cold water, juices, ice cream, and others). Due to the frequent and diverse utility of refrigerators, the chances of contamination would often be increased. Aesthetics showed as these handles were not cleaned for an extended period. Whereas serving counters were often found in good condition due to display and the main serving area, it was cleaned after every 15 to 20 minutes. However, APC was calculated as 3logCFU cm$^{-2}$.

The educated response was received from owners and food handlers for daily cleaning of the cafeteria.
and periodic cleaning of benches where food was being served. Despite their concern and proper reply for using new cleaning cloth every day, it was observed that cleaning cloth was in deplorable condition. We got three random samples for microbial analysis of those clothes. We found a high APC of 8.2 Log CFUcm\(^{-2}\) with mixed bacterial growth (MBG), along with yeast which was further isolated and reported in results.

The washing area was also found congested, but managed very well in some cafeterias. Others have poor conditions; hand washing soap was found at its proper location in some cafeterias, whereas detergents were often used to wash utensils and hands. We collected samples of null and washbasin, where workers used to place utensils for washing. APC for this parameter was calculated very high, as reported in Figure 1.

Hands of food handlers showed higher APC than previously reported as 7 log CFUcm\(^{-2}\)\(^{[13,39]}\) this showed poor hygienic practices that may lead to any gastrointestinal outbreak. While sampling it was found that food handlers were not aware of food safety and security. Few of the workers were observed practicing proper handwashing practices, but their clothes were mostly dirty. These workers were handling food with improperly washed hands, which seemed to be their common practice. During the survey, some of the food handlers had large hair and large nails. Dust observed in overgrown nails might be one of the causes of transmitting microbes in RTE foods prepared by those food handlers. Sometimes, it was also observed that a person having coughed and flu also process food with bare hands and without a mask. Most food handlers use aprons, but their aesthetics were poor, which ultimately increases food contamination chances.

*Pseudomonas* spp. Overgrowth was determined from many of the utensils used in one of the sampling points where one more visit was made for further investigation and resampling. It was found that the knife, cutting board, preparation surface, washing area, and refrigerator handle were having pseudomonas spp. All workers jointly shared contamination as all these places in the cafeteria, and one was ill. *Escherichia coli* was isolated in salads, sauce, Chaat, and other associated preparation surfaces; the same was isolated in fully cooked RTE foods, but their number was comparatively lower. After investigation, it was observed that tap water used in all food processing showed increased total and faecal coliforms.

5. Conclusion

In conclusion, it has been identified that water serves a role in hygiene; it is suggested to use clean water, preferably filtered for all sorts of processing and preparation of food materials. Washing of all associated food preparing and serving items should be conducted with great care. Properly cleaned utensils would minimize the risk of cross-contamination. The cleaning cloth should be used once every time and should not be reused, since it cannot attain a level of sterility after washing. Washing of hands after every step of processing should be promoted and hands should be properly sanitized to reduce the chances of contamination in the food chain. Display of RTE foods should be made in a closed area where surrounding dust, aerosols, and cross-hand contamination may be minimized.

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Disclosure statement

The authors declare that there is no conflict of interest.

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