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Full Length Research Paper

Bacteriological study of food in the Pakistan's peri-urban areas of Rawalpindi and Islamabad

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In order to ascertain the food quality for human consumption the bacteriological analysis was carried out in a prospective study in collaboration with National Institute of Health, Islamabad. The surveillance work was conducted during the months of July to August peak summer season to evaluate the objective parameters for assessment of the bacteriological and hygienic status of food being consumed in the peri-urban area of Islamabad and Rawalpindi. Out of a total of 91 samples, the various bacterial contaminants such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *fungal* species were isolated and identified using standard cultural and biochemical methods. It was observed that in total food samples, 25.5% were positive for *E. coli*, 16.6% for *S. aureus* and 11.1% for *Salmonella* species. This study highlighted the poor hygienic conditions for the food quality standards in the area of investigation. It was concluded that the food related endemic health concerns can be checked by taking appropriate preventive measures to forestall the outbreaks of gastroenteritis and food poisoning in the future as a serious health problem.

Keywords: Faecal coliforms, total viable count, total plate count.

INTRODUCTION

Growth of undesirable contaminating bacteria not only causes deterioration in sensory and organoleptic properties of food but can also cause illnesses. Most pathogenic microorganisms in food products are intestinal in origin, however some are found in the nasal passages, throat, on hair and on skin. Thus, food handlers are often a main source of contamination and cross contamination (Farooq et al., 2007).

Food poisoning due to food-borne pathogens is a major public health issue associated with food hygiene and overall food safety. Food-borne illnesses have been the focus of much public health attention over the past fifty years with microbiological contamination of food increasingly being recognized as a significant global problem. These illnesses are problem in both developed and developing countries. Diseases that spread through consumption of contaminated food or water principally in areas of poor sanitation include hepatitis A, hepatitis E and typhoid fever, diarrhea and dysentery (Light, 2000).

There are a number of methods that can be used to monitor the microbiological safety and quality of foods. The aerobic plate count (APC) is used as an indicator of the level of contamination by bacteria in a food product. Aerobic plate counts in food samples can sometime be useful to indicate quality, shelf life and post heat-processing contamination (GuaranTek Analytical Laboratories, 2003). Coliforms are a group of bacteria which comprised Gram negative, non spore forming aerobic rods that ferment lactose with the production of gas. There are several genera in this group including *Escherichia*, *Enterobacter or Klebsiella* (Khan et al., 2001). Coliform are present in faeces of all warm-blooded animals and humans and are used as a marker of unsanitary conditions or practices during production, processing or storage of food. The presence of coliforms in food, particularly processed meats, meat products and vegetables indicate the faecal contamination (Maturin

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Table 1. The level of microbial contamination in different samples of food.

S/N	Type of food	Number of sample	Fecal colifom	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	Raw food	11	6	9	6	4	4
2	Ready to eat food	14	4	6	4	3	2
3	Juices and liquid foods	21	3	6	4	4	1
4	Cooked food	45	10	15	9	4	3
Total		91	23	36	23	15	10

APC, Aerobic plate count.

and Peeler, 1998; Raloff, 2003; Jay, 1996). *Salmonella* is a typical member of the family Enterobacteriaceae, that have been recognized as causing enteric diseases for many years, and methods of control are well established. In addition, salmonellae remain the most important reported cause of food poisoning (Mead et al., 1999). *S. aureus* is another indicator of food contamination of processed foods. It could come from the skin, mouth, or nose of food handlers. It can be found in the air, dust, water and human faeces, and can be present on clothing and surfaces handled by people. Foods that frequently have a problem with staphylococcal food poisoning include milk and dairy products. *S. aureus* are capable of producing highly heat stable toxin that is the cause of staphylococcal food poisoning. Foods that require considerable handling during preparation and are kept at slightly elevated temperatures after preparation are frequently the ones involved in staphylococcal food poisoning (Fahed, 2003).

This study was undertaken to observe the quality of food in terms of its hygienic standards for human consumption and to assess the level of bacterial contamination in food being available to the community of Rawalpindi and Islamabad.

MATERIALS AND METHODS

This was an analytical and a cross-sectional study based on the evaluation of food hygienic quality in the peri-urban areas of Islamabad and Rawalpindi. We collected samples of routinely consumed food, ready to eat food, cooked and raw food along with some typical juices (Tables 2 to 4). All the samples were assigned laboratory codes to ensure the credibility and also to avoid any bias. The total of 91 samples ready to eat food, cooked, raw food and juices were analyzed for microbial contamination. All the samples were analyzed by using standard method to observe the load of microbial contamination. The samples were transported in sterile environment to the laboratory in the shortest possible time. The examination procedures carried out were bacterial total count, coliform count, *E. coli* and *S. aureus* assay along with screening for *Salmonella* sp.

Aerobic plate count

After homogenization, the sample for aerobic plate count was serially diluted in peptone water to 10^{-3}. The 10^{-1} to 10^{-3} dilutions were then plated on plate count agar (PCA) using a sterile pipette. This medium did not contain any inhibitors or indicators; it was mainly used to determine the total microbial content in food. The plates were incubated for 48 h (±4h) at 37°C (±1°C). The colony counts were made using the standard protocol and reported as colony forming units (cfu)/g.

To assess the level of microbial contamination in different food items, the colony-forming unit (cfu/ml) was obtained by multiplication with the dilution factor out of 0.05 ml of inoculum (20% of 1 ml). Subsequently conventional procedures were used for the culture and isolation of bacteria like coliform, fecal coliform or *E. coli*, *S. aureus* and *Salmonella* species in the given food sample upon the specific culture media generally in practice (Table 1).

RESULTS AND DISCUSSION

In this study, the contamination by the most commonly observed food pathogens such as *E. coli*, *S. aureus* and *Salmonella* and fungal species were studied to evaluate the level of food quality for human consumption.

Microbiological analysis of raw food

Different raw food samples were examined for APC, contamination by *E. coli*, *S. aureus* and *Salmonella*. The samples with lab nos. 90-F, 32-F and 96-F were found with no bacterial contamination and APC while only the samples with lab nos. 93-F, 96-F.9-F have APC. The samples with lab no. 127-F, 130-F, 157-F, 12-F, 13-F and 39-F were positive for faecal contamination and APC. The samples with lab nos. 12-F, 13-F and 39-F were positive for *S. aureus* while the samples 130-F, 12-F and 13-F were positive for *Salmonella* (Table 2). As these are raw food so the contamination may be due to washing with contaminated water or improper and unsafe handling.

Microbiological analysis of ready to eat food

Fourteen (14) samples of ready to eat food were examined for the APC, contamination by *E. coli*, *S. aureus* and *Salmonella*. In these samples, four samples were positive for faecal contamination and in six samples

Table 2. Microbial count in the raw food.

S/N	Sample lab number	Sample	Fecal coliform	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	90-F	Pea wheat blend(WFP)3Kg	Negative	Nil	Nil	Nil	Nil
2	93-F	Chicken butchery	Negative	1×10^3	Nil	Nil	Nil
3	96-F	Pea wheat blend(WFP)3Kg	Negative	8×10^2	Nil	Nil	Nil
4	127-F	Pea wheat blend(WFP)3Kg	Positive	5×10^4	4.8×10^3	Nil	Nil
5	130-F	Beef butchery	Positive	3×10^3	2×10^6	Nil	5×10^3
6	157-F	Chicken breast	Positive	1.4×10^5	4×10^2	40	Nil
7	9-F	Whole chicken	Negative	3×10^3	Nil	Nil	Nil
8	12-F	Chicken	Positive	4×10^6	2×10^6	1×10^4	2×10^6
9	13-F	Fish	Positive	5×10^6	1.8×10^6	4×10^2	2×10^3
10	32-F	Raw mutton	Negative	Nil	Nil	Nil	Nil
11	39-F	Fish	Positive	4×10^4	6×10^3	1.4×10^2	4×10^3

APC, Aerobic plate count.

Table 3. Microbial count in the ready to eat food.

S/N	Sample lab number	Sample	Fecal coliform	*APC*	*E. coli*	*S. aureus*	*Salmonella* sp.
1	99-F	Fresh Salad	Negative	Nil	Nil	Nil	Nil
2	111-F	High energy biscuits	Negative	Nil	Nil	Nil	Nil
3	123-F	Salad Tomatoes	Positive	4×10^4	2×10^4	Nil	2×10^3
4	132-F	Cucumber	Positive	2×10^6	1.4×10^6	4×10^3	Nil
5	142-F	High energy biscuits (A)	Negative	Nil	Nil	Nil	Nil
6	184-F	Salman apple jam	Negative	Nil	Nil	Nil	Nil
7	185-F	Salman honey	Negative	Nil	Nil	Nil	Nil
8	192-F	Salad	Negative	Nil	Nil	Nil	Nil
9	191-F	Green Salad	Negative	Nil	Nil	Nil	Nil
10	200-F	Ice-burg Salad	Negative	2×10^3	Nil	Nil	Nil
11	2-F	Chedder cheese	Negative	Nil	Nil	Nil	Nil
12	18-F	Tomato kachap	Negative	200	Nil	Nil	Nil
13	22-F	Fresh Salad	Positive	3×10^4	1×10^4	1×10^3	Nil
14	69-F	Fresh Salad	Positive	4×10^4	3×10^4	4×10^3	2×10^3

APC, Aerobic plate count.

APC was observed. Three samples were positive for *S. aureus* and two were positive for *salmonella* sp. (Table 3). The bacterial contamination is mostly observed in salads which may be due to washing with contaminated water or improper and unsafe handling.

Microbiological analysis of juices and liquid food

The 21 samples of different brand of juices, milk and soft drinks were processed for APC, *E. coli*, *S. aureus* and *Salmonella*. There was no bacterial contamination found in food samples with lab. Nos. 94-F, 95-F, 97-F, 98-F, 106-F, 34-B, 140-F, 145-F, 147-F, 187-F, 16-F, 20-F, 21-F, 41-F, 43-F and 44-F but the samples with lab nos. 18-B, 19-B, 23-B and 186-F were positive for faecal coliform, *E. coli* and *S. aureus* (Table 4). The sample 186-F was the only sample that was positive for *Salmonella*.

There was no contamination in the sealed juices and milk packs which showed that they were processed and manufactured in good hygienic conditions. Only open juices and raw milk showed faecal contamination that may be due to the exposure with unclean open environment and use of contaminated water and unhygienic handling.

From the Suguna et al. (2011) results, all the juice samples were found to be contaminated by bacteria [(total plate counts (TPC) and APC), yeasts and molds (TYC and TMC)]. The presence of microorganisms in the juice samples might be attributed to the pre- and post-harvest storage conditions as well as to improper handling during transportation of the fruit. Overall

Table 4. Microbial count in the juices and liquid foods.

S/N	Sample lab number	Sample	Faecal coliform	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	94-F	Mango (cold kitchen)	Negative	Nil	Nil	Nil	Nil
2	95-F	Leman squash (shezan1000 ml)	Negative	Nil	Nil	Nil	Nil
3	97-F	Drinka mango drink 250 ml	Negative	Nil	Nil	Nil	Nil
4	98-F	Drinka apple drink 250 ml	Negative	Nil	Nil	Nil	Nil
5	106-F	Strawberry seedless sauce	Negative	Nil	Nil	Nil	Nil
6	18-B	Mango juice (open)	Positive	5×10^6	6×10^6	2×10^6	Nil
7	19-B	Milk shake juice (open)	Positive	7×10^6	4.6×10^6	1.8×10^6	Nil
8	23-B	Soda water	Negative	5×10^4	4×10^4	6×10^3	Nil
9	33-B	Chocolate sauce	Negative	6×10^2	Nil	Nil	Nil
10	34-B	Green color (food color)	Negative	Nil	Nil	Nil	Nil
11	140-F	Shark Energy drink	Negative	Nil	Nil	Nil	Nil
12	145-F	Sheezan lemon squash	Negative	Nil	Nil	Nil	Nil
13	147-F	Strawberry fruit sauce with pieces	Negative	Nil	Nil	Nil	Nil
14	186-F	Raw milk	Positive	5×10^6	4×10^6	2×10^6	2×10^6
15	187-F	Boiled milk	Negative	Nil	Nil	Nil	Nil
16	16-F	Beef sausage	Negative	1.6×10^3	Nil	Nil	Nil
17	20-F	Spirit	Negative	Nil	Nil	Nil	Nil
18	21-F	Cocacola	Negative	Nil	Nil	Nil	Nil
19	41-F	Haleeb milk pack	Negative	Nil	Nil	Nil	Nil
20	43-F	Alphurs milk pack	Negative	Nil	Nil	Nil	Nil
21	44-F	Nestle milk pack	Negative	Nil	Nil	Nil	Nil

APC, Aerobic plate count.

Table 5. Microbial count in baked and bakery food.

S/N	Sample lab. number	Sample	Fecal coliform	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	103-F	Cake	Negative	Nil	Nil	Nil	Nil
2	104-F	Sawyyian	Negative	Nil	Nil	Nil	Nil
3	26-B	Patties	Negative	Nil	Nil	Nil	Nil
4	27-B	Bread	Negative	2×10^3	Nil	Nil	Nil
5	153-F	Pizza	Negative	80	Nil	Nil	Nil
6	23-F	Tifta custard	Positive	3×10^3	Nil	Nil	Nil
7	68-F	Date cake	Negative	Nil	Nil	Nil	Nil

APC, Aerobic plate count.

comparison, higher microbial load was recorded in the juice samples collected from street vendors compared to those prepared in the laboratory.

Microbiological analysis of cooked food

There were 45 samples of cooked food which were further categorize into five different groups that is, baked and bakery food, cooked food having chicken as ingredient, cooked food having vegetable as ingredient, cooked food having rice as ingredient and fried cooked food.

Microbiological analysis of baked and bakery food

In this category seven samples were analyzed. In these samples, the samples with lab nos. 103-F, 104-F, 26-B and 68-F were free from bacterial contamination while in samples 27-B, 153-F and 23-F APC was observed and the highest count was in 23-F, that is, 3×10^3. 23-F was the only sample that

Table 6. Microbial count in cooked food having chicken as ingredient.

S/N	Sample lab. number	Sample	Fecal coliform	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	91-F	Roasted chicken (N.C.S)	Negative	Nil	Nil	Nil	Nil
2	20-B	Chicken Karahi	Negative	1×10^3	Nil	Nil	Nil
3	25-B	Chicken Tikka	Positive	6×10^4	4×10^4	2×10^3	Nil
4	29-B	Chicken Karahi	Negative	Nil	Nil	Nil	Nil
5	30-B	Chicken Karahi	Negative	Nil	Nil	Nil	Nil
6	36-B	Chicken Roll	Negative	3×10^6	Nil	Nil	Nil
7	15-F	Grill Chicken	Negative	Nil	Nil	Nil	Nil
8	30-F	Chicken jalfrezi	Negative	Nil	Nil	Nil	Nil
9	35-F	Chicken achar kurry	Negative	Nil	Nil	Nil	Nil
10	61-F	Steam roast	Negative	Nil	Nil	Nil	Nil
11	66-F	Chicken steak	Negative	Nil	Nil	Nil	Nil
12	70-F	Chicken palak	Negative	Nil	Nil	Nil	Nil

APC, Aerobic plate count.

Table 7. Microbial count in cooked food having vegetable as ingredient.

S/N	Sample lab. number	Sample	Fecal coliform	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	100-F	Stir fried vegetable	Negative	Nil	Nil	Nil	Nil
2	102-F	Vegetable Bhujia	Positive	1×10^4	2×10^4	Nil	Nil
3	24-B	Sabzi	Negative	1×10^3	Nil	Nil	Nil
4	31-B	Palak	Negative	Nil	Nil	Nil	Nil
5	120-F	Aloo Tori vegetable	Positive	2.6×10^4	6×10^3	Nil	Nil
6	63-F	Vegetable rice	Negative	Nil	Nil	Nil	Nil

APC, Aerobic plate count.

was positive for the faecal contamination (Table 5).

Microbiological analysis of cooked food having chicken as ingredient

Twelve (12) samples were analysed in which only one sample with lab no. 25-F was positive for faecal coliform and in tree samples that is, 20-B, 25-B and 36-B APC was observed and the highest count was in the sample 25-B that is, 6×10^4 (Table 6).

Microbiological Analysis of cooked food having vegetable as ingredient

Six cooked vegetables were analyzed and in these six samples three that is, 100-F, 31-B and 63-F were free from bacterial contamination. In three samples, APC was observed and also two samples that is, 102-F and 120-F were positive for faecal coliform (Table 7).

Microbiological analysis of cooked food having rice as ingredient

Nine samples were analyzed in which only two samples that is, 21-B and 64-F were positive for faecal coliform, *E. coli*, *S. aureus and Salmonella* sp. (Table 8).

Microbiological analysis of fried cooked food

A total of 11 samples of fried cooked food were analyzed. In these samples only four samples that is, 28-B, 118-F, 119-F and 124-F were positive for faecal coliform and *E. coli* and also APC was observed in these samples. Only one samples with lab no. 28-B was positive for *S. aureus* and sample with lab no. 124-F was positive for *Salmonella* sp. (Table 9).

In the cooked food, contamination may occur due to the inadequate cooking, washing with contaminated water, unhygienic handling and delivery in contaminated dishes, which demonstrated the poor sanitary conditions. Nichols

Table 8. Microbial count in cooked food having rice as ingredient

S/N	Sample lab. number	Sample	Fecal coliform	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	101-F	Yakhni Pullao	Negative	Nil	Nil	Nil	Nil
2	21-B	Ublish Pullao	Positive	4×10^4	3×10^4	2×10^3	3×10^3
3	125-F	Rice	Negative	Nil	Nil	Nil	Nil
4	24-F	Boiled rice	Negative	Nil	Nil	Nil	Nil
5	25-F	Yakhni pilaow	Negative	Nil	Nil	Nil	Nil
6	26-F	Zeera pilaow	Negative	Nil	Nil	Nil	Nil
7	64-F	kheer	Positive	2.5×10^7	1.5×10^6	2×10^3	2×10^6
8	65-F	Eggs Fried rice	Negative	Nil	Nil	Nil	Nil
9	67-F	Loki kheer	Negative	Nil	Nil	Nil	Nil

APC, Aerobic plate count.

Table 9. Microbial count in fried cooked food.

S/N	Sample lab. number	Sample	Fecal coliform	APC	*E. coli*	*S. aureus*	*Salmonella* sp.
1	28-B	Kabab	Positive	5×10^6	3×10^6	5×10^5	Nil
2	118-F	Dal Moong	Positive	3×10^4	2.4×10^4	Nil	Nil
3	119-F	Dal Moong+Dal Chuna	Positive	3×10^4	2×10^4	Nil	Nil
4	121-F	Fry Meat	Negative	Nil	Nil	Nil	Nil
5	122-F	Samosa	Negative	Nil	Nil	Nil	Nil
6	124-F	Chema	Positive	3×10^4	2.2×10^4	Nil	1.6×10^3
7	177-F	Shamikabab	Negative	Nil	Nil	Nil	Nil
8	197-F	Namkeen Goasth	Negative	Nil	Nil	Nil	Nil
9	203-F	Fish	Negative	Nil	Nil	Nil	Nil
10	62-F	Mutton kurma	Negative	Nil	Nil	Nil	Nil
11	92-F	Mushroom	Negative	Nil	Nil	Nil	Nil

APC, Aerobic plate count.

(2002) also showed that pathogenic bacteria including *S. aureus*, *E. coli* and *Salmonella* in restaurants may be transferred to the cooked foods by its contaminated staffs' hands or dishes. Tavakoli and Riazipour (2008) observed in their study, the possibility for the cooked foods to be contaminated with coliforms and pathogenic bacteria including *E. coli* and *S. aureus*, as 50% of the 216 samples examined in their were study indicated to have coliforms contamination. The *S. aureus* and *E. coli* contamination was also found in 14.2% and 12.6% of the examined samples, respectively. Their findings have shown conformity to our results.

Salek (2000) conducted an assessment on 100 samples taken from meat foods offered in clinical centers of Shahid Beheshti University of Medical Sciences. Mean total bacterial count were 2.04×10^5, 2.16×10^2, 2.45×10^4 and 2.25×10^4 cfu/g in samples of grilled ground meat, grilled chicken, chicken and hamburger, respectively. In this study also, different cooked food such as meat and chicken contained the bacterial contamination. They also found 28 out of 61 samples (46%) positive for *S. aureus*, which showed highest contamination than our results (16.6%). It may be due to the poor sanitary conditions in restaurants of this university.

According to Adolf and Azis (2012), the high level of microbial contamination could come from improper sanitation practices at the canteen during the processing and selling period. As reported by Ahmed et al. (2008), lack of good sanitation practices and proper storage will increase microbial contamination. Microbial contamination can also be caused by microbes that naturally grow at those foods. Moreover, according to Easa (2010), Udo et al. (2009), Uzeh et al. (2009), Elmacioglu et al. (2010), Bukar et al. (2010) and Ahmed et al. (2008), it was found that various pathogenic bacteria existed in food contained of meat, vegetable, flour, spices, tomato and egg.

Conclusion

In food samples, highest contamination was found in raw

food followed by cooked food and juices. It is evident that people of this study area are at high risk of food diseases caused by microorganisms. Good food practices, such as adequate cooking, hygienic food processing and handling can greatly minimize the risk of food borne diseases.

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