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Microbiological Evaluation of Different Types of Branded and Non-branded Ready-to-Eat Snacks Sold in Elementary Schools of District Peshawar, Pakistan

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ABSTRACT

The lack of hygiene in the preparation and packing of ready-to-eat foods is fast becoming a serious public health concern, especially for school children. The intervention of different pathogenic microorganisms in these foods poses the risk of foodborne disease outbreaks. The current study was designed to assess bacterial contamination in different types of ready-to-eat (branded and non-branded) snacks purchased from September 2021 to December 2021 in various elementary schools of Peshawar, Pakistan. A total of 20 samples were collected and analyzed using the pour plate method for total plate count (TPC). Moreover, the multiple fermentation tube method was used for total coliforms (TC) and fecal coliforms (FC). *Escherichia coli* isolates were identified using *E. coli* O157:H7 latex test reagent kit Pro Lab, Canada. The results revealed contamination by TPC (6.67%), TC (40%), FC (33.33%), and *E. coli* (20%) in the samples. These values were higher than the permissible limits set by Food and Agriculture Organization (FAO). Hence, it was concluded that there is a practice gap in food safety knowledge among ready-to-eat food vendors. The vendors are usually untrained and lack the knowledge of proper hygiene and food handling procedures. It is suggested here that the government should pay special attention towards improving public awareness regarding food safety and quality of ready-to-eat foods sold in Peshawar, Pakistan.

Keywords: coliforms, *E. coli,* fecal coliforms (FC), foodborne, ready-to-eat, snacks, total coliforms (TC), total plate count (TPC)

1. INTRODUCTION

Food safety is a fundamental issue and it requires efforts to prevent its contamination by biological and chemical agents, that is, the substances that damage, injure, and threaten human health [1]. Ready-to-eat foods may be raw or baked, ready for immediate consumption at the point of sale, and may be consumed without further care [2]. Dried snacks such as chips, cookies, roasted nuts, and muesli are common snack products available in grocery stores, mega shops, and even in remote areas, all over the world. These are mostly enjoyed during journeys, picnics, and even as school tiffin. Cooked and salted potato parts combined with various forms of flavoring ingredients, including spices, herbs, cheeses, natural or artificial flavors, and additives are used for potato chips preparation [3].

The World Health Organization (WHO) reported that every-day, more than 5,000 children die from the ingestion of polluted food and water, globally. In all parts of the world, food borne diseases are

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prevalent and their effects on human well-being are immense, leading to substantial economic losses. The rate of occurrence of foodborne diseases is growing as well. Around one-third of the population suffers from foodborne diseases every year in developed countries, while the situation is poorer in developing countries due to overcrowding, poverty, insufficient sanitary circumstances, and poor common hygiene [4].

The nutrition process includes the ingestion, digestion, absorption, and metabolization of nutrients essential to the normal function of the body [5]. There has been a steady growth in the consumption of snacks and chips over the past 15 years. This development is due to the urban population's increased pace of life. Snacks and chips are used as alternatives to main dishes, and so as non-alcoholic or low-alcohol beverages for consumption. Gorgeous organoleptic possessions, the capacity to rapidly reduce hunger, and the comfort of consumption are the key requirements that customers place on snacks and chips. Food products containing higher amounts of proteins and dietary fibers, which are sources of significant nutritional worth, are demanded by the consumers [6]. Snacks are primarily processed foods made up of potatoes and other vegetables. Due to their mouthwatering taste and easy availability in the market, corn and potato snacks are now very popular globally, particularly among young children [7].

Foodborne diseases are caused by the ingestion of bacteria, toxins, and other microorganisms present in the food. Possible health threats are associated with food contamination by *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas* species, and *Proteus* spp. These threats arise due to the unsafe and unhygienic food preparation practices of the vendors. The inconsistency of the products they use including their hands and fabric, cross-contamination between dishwater, surfaces for food preparation, and the food itself [8]. The majority of hospital deaths are due to bacterial agents, although half of the foodborne illnesses are caused by viruses. Pathogens cause a wide variety of infections or intoxications, such as enteric complications, hemorrhagic colitis, stomach pain, fever, meningitis, bloodstream infection, joint infection, paralysis, kidney failure, and miscarriage. According to the World Health Organization (WHO), foodborne and water-borne diseases globally kill around 1.9-2.2 million people, annually [9]. Microorganism-contaminated potato chips can cause health-related problems. Fungus-contaminated nuts and related toxins can cause liver damage, cancer, and abortion [3].

Therefore, the continuous assessment of the microbiological quality of food is very important. The current study was designed to identify the existence and number of bacteria by total plate count (TPC), total coliforms (TC), fecal coliforms (FC), and *E. coli* in various branded and non-branded ready-to-eat snacks purchased in various elementary schools (government and private) located in Peshawar, Pakistan.

2. MATERIALS AND METHODS

2.1. Samples Collection

A total of twenty (20) different types of branded (potato and corn-based) and non-branded snack samples were collected from September 2021 to December 2021 from various elementary schools (government and private) located in Peshawar, Pakistan. All samples were transferred in sterile food bags to Food Microbiology Lab at PCSIR, Peshawar and analyzed microbiologically

for TPC, TC, FC, and *E. coli*, as per the method described by Food and Agriculture Organization (FAO) with slight modification [10].

2.2. Sample Preparation

About 50 g of each sample was blended in 450 ml Butterfield's phosphate buffer using High-Density Polyethylene (HDPE) bags. Stomacher (Model 400 Circulator, England) was used to homogenize the sample for 2 mins, making 10^{-1} dilution. Serial dilutions (10^{-2} to 10^{-4}) of the snack samples were made to satisfy the calculation's requirement of colonies per gram (CFU/g) [11].

2.3. Microbiological Analysis

2.3.1. Total Plate Count (TPC). All samples were diluted and aliquots of 1 ml were inoculated to plate count agar (PCA, OXOID) in laminar flow hood (Streamline Lab Products. EN-1822.1, Pakistan) to each duplicate petri dish for the counting of TPC. The plates were incubated in an incubator (Memmert Germany, DIN-40050-IP20, FVR-831537) at 35ºC for 48 h. After incubation, the colony was counted by colony counter (Stuart Scientific, Bibby Sterlin Ltd UK) and the result was expressed as CFU/g.

2.3.2. Total Coliforms (TC) and Fecal Coliforms (FC). TC and FC count were determined using the most probable number (MPN) method [10], with some modifications. Briefly, the prepared sample for TPC count in different dilutions was inoculated in three sets of lauryl tryptose broth (LTB) and incubated at 35ºC for 48 h. After incubation, the growth, acidic reaction, and appearance of gas in LTB were used for a presumptive count. Growth or gas production in brilliant green bile broth was used as the confirmatory test for TC. Simultaneously, FC were obtained in *E. coli* (EC) broth tubes incubated for 24 h at 44.5ºC.

2.3.3 *E. coli* Detection. The presence of *E. coli* was determined based on the method described by FAO [10] using positive tubes of LTB medium. A loopful from these tubes was transferred into a mug containing EC broth and the tubes were incubated for 18-24 h at 44.5ºC. The tubes were observed for growth, acidic reaction, and appearance of gas. *E. coli* colonies were grown on eosin methylene blue (EMB) agar and observed as (dark centered with metallic sheen). The typical colonies were confirmed by *E. coli* O157:H7 latex test reagent kit (Pro Lab. Canada).

3. RESULTS

3.1. Microbial Contamination in Potato Snacks (Branded)

Specific legends were assigned to all collected samples. TPC in the sample (HPS) was found to be 3.22×10^4 CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in the sample (SS) was 4.74×10^4 CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in the sample (MS) was 4.9 $\times10^3$ CFU/g, TC and FC were 93 MPN/g, while *E. coli* were 23 MPN/g. TPC in the sample (TK) was TPC 3.7 $\times10^3$ CFU/g, TC was 43 MPN/g, FC was 15 MPN/g, and *E. coli* was <3MPN/g. In sample (SSN) TPC was 1.27 $\times10^4$ CFU/g, TC, FC, and *E. coli* were <3MPN/g. TPC in the sample (MPS) was 1.78 $\times10^4$ CFU/g, while TC, FC, and *E. coli* were <3MPN/g. TPC in the sample (BS) was 4.3 $\times10^4$ CFU/g, TC was 28 MPN/g, FC was 15 MPN/g, and *E. coli* was 07 MPN/g (Table 1).

Table 1. Microbiological Analysis of Potato Based Branded Snacks

Sample ID	TPC (CFU/g) *10^4-10^5	TC (MPN/g) *<3	FC (MPN/g) *<3	*E. coli* (MPN/g) *<3
HPS	3.22×10^4	<3	<3	<3
SS	4.74×10^4	<3	<3	<3
MS	4.9×10^3	93	93	23
TK	3.7×10^3	43	15	<3
SSN	1.27×10^4	<3	<3	<3
MPS	1.78×10^4	<3	<3	<3
BS	4.3×10^4	28	15	07

TPC = Total Plate Count; TC = Total Coliform; FC = Fecal Coliform; * FAO Standards; CFU = Colony-Forming Unit; MPN = Most Probable Number

3.2. Microbial Contamination in Corn Snacks (Branded)

TPC in sample (HS) was found 3.8 $\times 10^2$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in sample (KKS) was 3.3 $\times 10^4$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in sample (MKS) was 2.94 $\times 10^4$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in sample (HS) was 3.4 $\times 10^3$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in sample (KSS) was 2.0 $\times 10^2$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in sample (FS) was 9.0 $\times 10^1$ CFU/g, while TC, FC, and *E. coli* were <3MPN/g. TPC in sample (FN) was 2.1 $\times 10^2$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g (Table 2).

Table 2. Microbiological Analysis of Corn Based Branded Snacks

Sample ID	TPC (CFU/g) *10^4-10^5	TC (MPN/g) *<3	FC (MPN/g) *<3	*E. coli* (MPN/g) *<3
HS	3.8×10^2	<3	<3	<3
KKS	3.3×10^4	<3	<3	<3
MKS	2.94×10^4	<3	<3	<3
HS	3.4×10^3	<3	<3	<3
KSS	2.0×10^2	<3	<3	<3
FS	9.0×10^1	<3	<3	<3
FN	2.1×10^2	<3	<3	<3

TPC = Total Plate Count; TC = Total Coliform; FC = Fecal Coliform; * FAO Standards; CFU = Colony-Forming Unit; MPN = Most Probable Number

3.3. Microbial Contamination in Local Snacks (Non-Branded)

TPC in sample (FR) was found 2.4 $\times 10^2$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in sample (VN) was 1.3 $\times 10^2$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g. TPC in sample (SS) was 1.8 $\times 10^4$ CFU/g, while TC was 7 MPN/g, FC, and *E. coli* were <3 MPN/g. TPC in sample (WS) was 2.56 $\times 10^2$ CFU/g, while TC was 11 MPN/g, FC and *E. coli* were 9 MPN/g. TPC in sample (JS) was 1.96 $\times 10^5$ CFU/g, while TC was 7 MPN/g, FC was 4 MPN/g and *E. coli* was <3 MPN/g. TPC in sample (GS) was 8.3 $\times 10^2$ CFU/g, while TC, FC, and *E. coli* were <3 MPN/g (Table 3).

Table 3. Microbiological Analysis of Local (Non-Branded) Snacks

Sample ID	TPC (CFU/g) *10^4-10^5	TC (MPN/g) *<3	FC (MPN/g) *<3	*E. coli* (MPN/g) *<3
FR	2.4×10^2	<3	<3	<3
VN	1.3×10^2	<3	<3	<3
SS	1.8×10^4	07	<3	<3
WS	2.56×10^2	11	09	09
JS	1.96×10^5	07	04	<3
GS	8.3×10^2	<3	<3	<3

TPC = Total Plate Count; TC = Total Coliform; FC = Fecal Coliform; * FAO Standards; CFU = Colony-Forming Unit; MPN = Most Probable Number

3.4. Overall Microbial Contamination in Snacks

According to the FAO standards, TPC was high in *n* = 01 (6.67%) among the analyzed samples. TC was high in *n* = 06 (40%), while FC was high in *n* = 05 (33.33%). *E. coli* was found to be high in *n* = 03 (20%) (Figure 1).

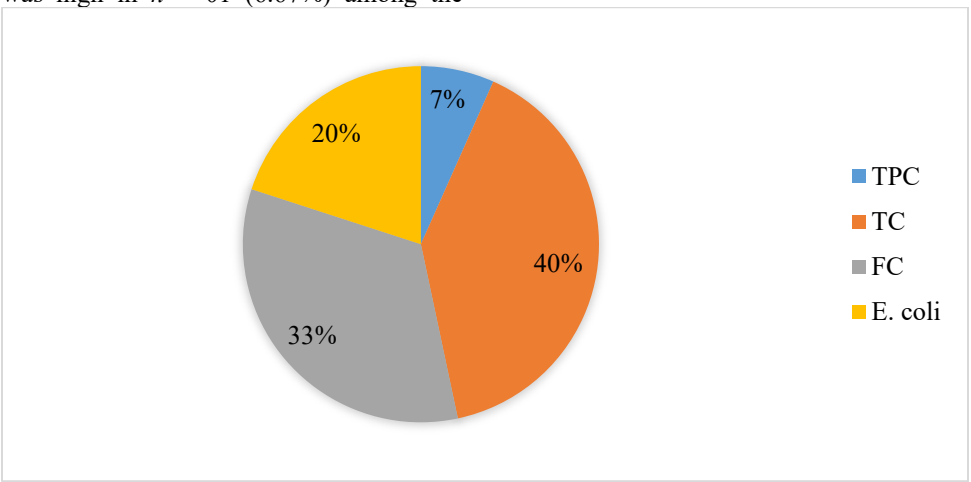

Figure 1. Overall Microbial Contamination in Snacks Samples

4. DISCUSSION

In this study, a total of 20 different branded and non-branded snack samples were analyzed for TPC, TC, FC, and *E. coli*. It was found that the potato branded snack samples had TPC below the permissible limits. The TC of sample (MS) was 93 MPN/g, of sample (TK) was 43 MPN/g, and of sample (BS) was 28 MPN/g, which were all higher than the permissible limits of FAO. FC count was also found to be higher than the permissible limit. Sample (MS) had an FC count of 93 MPN/g, sample (TK) had an FC count of 15 MPN/g, and sample (BS) had an FC count of 15 MPN/g. *E. coli* was also found to be higher than permissible limits. In sample (MS), its count was 23 MPN/g and in sample (BS) its count was 07 MPN/g. The high growth counts found in this study could be attributed to a variety of factors such as inadequate storage facilities,

vendor's personal hygiene, and lack of proper sanitation [12]. The results obtained from the microbiological assessment of school-vended food samples showed that most food items conformed to the limits of satisfactory and acceptable microbiological quality [13].

TC bacteria count was found to be the highest among all the samples (40%), followed by FC (33.33 %), *E. coli* (20%), and TPC (6.67%) counts. These results may be due to poor sanitary practices by food personal and could be an indication of possible fecal contamination [14]. These results also indicated poor sanitary control and practices [15]. Bacterial isolates in snack samples can be responsible for newborn meningitis and infantile diarrhea [16].

On the other hand, TPC count was found within the permissible limits in all samples of corn-based branded snacks, whereas no contamination regarding TC, FC, and *E. coli* was found in these samples. Among all samples of local (non-branded) snacks, TPC count was found to be higher than the permissible limits in sample SS and JS, while in the remaining samples contamination was found within the permissible limits [10]. TC count was found to be higher in sample (WS), sample (SS), and sample (JS). FC count was also found to be higher in samples (WS) and sample (JS). *E. coli* was also found to be higher than permissible limits in sample (WS).

Some of the results regarding microbiological contamination in different branded and non-branded snacks from various elementary schools indicated poor food hygiene procedures and sanitary conditions. These results are in line with the results of the studies conducted in Ethiopia [17] and Bangladesh [18] in which TPC counts of SVFs were in the range of 1×10^4-1.86×10^5 CFU/g and 1.2×10^3-4.2×10^9 CFU/g, respectively. The majority of merchants handle these snacks according to good hygiene and sanitation principles, such as buying food from a safe source, preventing bacteria from entering food, and preventing the multiplication of bacteria in the food. Snack vendors may not have received adequate training regarding the importance of hygiene and sanitation principles in food management [19, 20]. There is a need to establish a regular monitoring program as well as developing an awareness plan among vendors at schools to improve their understanding regarding microbiological safety. Moreover, various intervention programs should be presented to the vendors.

4.1. Conclusion

The current study concludes with the fact that some of the most common types of ready-to-eat snacks sold in schools in District Peshawar are contaminated. They do not meet the requisite quality and safety standards. TC, FC, and *E. coli* are known to cause gastroenteritis and are possible enteric pathogens. As a result, street foods pose a major health risk to patrons and efforts to minimize contamination levels in street food are recommended. The presence of foodborne pathogens is a serious health risk. The ready-to-eat food handlers and processors should be trained on appropriate hygiene practices for the production, processing, storage, and handling of their food items.

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