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A Study on Microbial Contamination of Foods Exposed to Multiple Environments

[1] Dan-Bee KIM [2] Seong-Soo CHA

[1. First Author] Department of Food Science and Service, College of Bio-Convergence, Eulji University, Korea.
[2 Corresponding Author] Department of Food Science and Service, College of Bio-Convergence, Eulji University, Korea. Tel: +82-31-740-7274, E-mail: sscha@eulji.ac.kr

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Abstract

In this study, general bacterial counts and coliform counts, which are hygienic indicator microorganisms, were tested for candy, chocolate, and jelly which are easily available and enjoyed around. After dropping each sample on the desk, indoors, and outdoors, it is immediately collected, or washed and collected to confirm the myth of the 3-second rule. Immediately after removing the wrapping paper, each sample was dropped on the desk, indoors, and outdoors, and after 3 seconds from the moment of contact with the surface, and then collected in a sample bag using sterilized sanitary gloves. After the same operation, each sample was rinsed for 5 seconds using sterilized sanitary gloves and sterilized distilled water, and then collected in a sample bag. The number of bacteria detected in non-washing candies was 41 CFU/g at outdoor and the number of bacteria detected in non-washing chocolate was 76 CFU/g at outdoor. The number of bacteria detected in non-washing jellies was 79 CFU/g at outdoor. Coliform group was not detected in all samples. This showed good results at the level of m = 10,000 or less, which is an allowable value suggested in the Food Code. Also, effect of washing on contaminated food was confirmed. This result is remarkably low compared with the microorganism specimens shown in Food Code, and it is confirmed that contamination occurs but not high value. Therefore, the myth of the 3-second rule is true compared to the figures based on Food Code. However, it showed the characteristics of bacteria that could survive and cross-contaminate on dry food surfaces and emphasized the importance of hygiene through food contact to unsanitary surfaces to minimize the risk of food poisoning.

Keywords: Bacteria, coliform, cross-contamination, three-second rule, bacterial transfer, food contact surface, Korea Food Code.

Major classification: Food hygiene science.

1. Introduction

In terms of food hygiene, it is customary to discard food that has reached unsanitary surfaces. However, those who support the three-second rule hypothesis argue that even if food has been dropped to an unsanitary surface, it is not exposed to any bacteria contamination as long as it is picked up within three seconds. While the three-second rule is not socially accepted in public arenas such as restaurants, it is likely to be tolerated in private situations like at home. Nevertheless, the three-second rule remains as a mere hypothesis, rather than a social fact, because it is not supported by sufficient scientific evidence.

According to the data provided by the Ministry of Food and Drug Safety of South Korea, the number of food poisoning cases in 2013 was 235 with 4,958 patients. In 2014, the number of food poisoning cases increased to be 349 with 7,466, and has been steadily increasing until 2016. In 2017, the number of reported case slightly decreased

to 336 with 5,649 patients. However, as of September 17, 2018, 361 cases of food poisoning occurred and 8,985 patients were recorded. The most common causes of food poisoning were pathogenic *Escherichia coli* (10,348), followed by *norovirus* (6,863). The cause of 752 cases of food poisoning which affected 5,110 patients has not been identified (Korea Ministry of Food and Drug Safety, 2018).
Unlike adults, Enteritis caused by ingestion of contaminated food or bacterial diarrhea may result in physical loss particularly in infants and young children. Thus, it is important to investigate and control the microbiological contamination status of complete products and raw materials (Jung, W. Y. et al., 2010). Therefore, we propose an experimental research which investigates the effects of bacterial contamination by using snacks that children enjoy. We study children because children have weaker immune system than adults, and thus are more susceptible to diseases if they picked up food which has been dropped.

Bacteria can survive when in contact with food, which can lead to cross contamination when in contact with unsanitary surfaces (Dawson, Han, Cox, Black, & Simmons, 2007). Research on cross-contamination has been done through a variety of settings. For example, Patrick, Findon, and Miller (1997) found that hands contaminated with bacteria could also contaminate skins or other objects even if they are washed. However, when hands were dry, bacterial contamination was significantly reduced compared to wet hands. Thus, water seems to play an important role in transferring bacteria to the food surfaces. Places such as sinks, and objects like wet cloths and scrubbers were identified as the causes of cross contamination (Scott & Bloomfield, 1990; Dawson et al., 2003). The survey in the U.S. of 200 households, a large number of aerobic bacteria were found in 49% of the food contact surfaces where pathogenic bacteria is likely to grow (Scott et al., 1982). Several factors can contribute to the rate of bacterial transfer from the contacted surface to food: the composition of the food and the type of contact surface, bacterial residence time and contact time. The three-second rule refers that when food that has been dropped on unsanitary surfaces and picked up within three seconds, the food is not contaminated. On the other hand, according to a study by the University of Maine, a five-second rule of thumb could reduce food waste and even improve children's immune systems if they eat dropped foods.
Therefore, the purpose of this study is to investigate the effect of food contact time on contaminated surfaces on bacterial transfer and the effect of food composition. It also examines how much bacteria could be removed when rinsing contaminated food with water. The microbiological standards for candy products and processed chocolate products in Korea are as follows: n = 5, c = 2, m = 10,000, and M = 50,000.

2. Research design

2.1. Sample selection

The samples used in this study were three different types of candies, chocolates, and jellies. As stated in the Food Code, five samples manufactured on the same date have been purchased from a convenient store in Michuhol-gu in September, 2018. Each sample was stored in a cool, dry place.

2.2. Microbial analysis method

Bacterial count and coliform group were hygienic indicator bacteria, and they were tested in accordance with the microbiological test method during the general experiment of food revolution. The confirmed microbial population results are expressed as CFU/g.

2.3. Experiment settings

Each sample was dropped outdoor without the packaging paper, on the floor of the room and on the desk. Immediately 3 seconds after the sample touched the surface, it was collected in a sample bag using sterilized sanitary gloves. After repeating the same procedure on every sample, each sample was rinsed with sterilized distilled water for five seconds, and then was collected in a sample bag. Each sample was repeatedly operated in five times. Additionally, a control experiment was conducted to examine the colony count of bacteria and coliform group before the experimenting samples to be analyzed as zero. The control experiment aimed to reduce external errors that might occur during the experiment. In the control experiment, the samples from which the wrapping paper was removed were aseptically collected in a sample bag. The rest of the experimental methods remained the same.

2.4. Preparation of test liquid

All samples were aseptically collected, recovered, separately placed in a sample bag (3M, USA), diluted with sterile physiological saline (BNF Korea, Korea) at a ratio of 1:10 (w / v). The samples were homogenized for 20 seconds using a homogenizer (Gamme Bag Mixer 400, Interscience, France), and in accordance with the Guidelines of the International Commission on Microbiological Specifications for Foods (ICMSF). Five samples of the same product with the same manufacture date were repeatedly tested.

2.5. Standard plate count quantitative analysis method

The test was carried out in accordance with Article 7. General Testing Methods of Food Code, 4. Microorganism Test Method, 4.5 Bacterial Count, 4.5.1 Standard plate count, and b. Dry film method. According to the Food Code, 1 ml of the homogenized test solution and 1 ml of the 10-fold dilution were inoculated into two sets of Aerobic Count Plate (3M, USA) for measurement of Standard plate count, and the cells were incubated at 35 ± 1 ° C for 48 ± 2 hours. The number of red colonies was counted by taking the plate from which 30-300 colonies were generated. The average number of colonies was multiplied by the dilution factor and Standard plate count was measured. The calculation of the number of bacteria and the report of the description were made according to the 4.5.1 Standard plate count, a. Standard plate method and 3) Number of bacteria description.

2.6. Coliform group quantitative analysis method

The test was carried out in accordance with Article 7. General Testing Methods of Food Code, 4. Microorganism Test Method, 4.7 Coliform group, 4.7.2 Quantitative analysis, and c. Dry film method. According to the Food Code, 1 ml of the homogenized test solution were inoculated into two sets of *E. coli* / Coliform Count Plate (3M, USA) for measurement of coliform counts and the cells were incubated at 35 ± 1 ° C for 24 ± 2 hours. The number of colonies forming bubbles around the red colonies was counted by selecting the plates that produced 30 to 300 colonies. The average number of colonies was multiplied by the dilution factor and the number of coliform was measured. The calculation of the number of bacteria and the report of the description were made according to the 4.5.1 Standard plate count, a. Standard plate method and 3) number of bacteria description.

3. Result

3.1. Standard plate count distribution status

Although the presence or absence of Standard plate count is not directly related to any physical harm to the human body, it can be used as an indicator for determining the sanitary condition of food. The number of bacteria detected in non-washed candies was 41 CFU/g outdoor, the indoor average was 1 CFU / g, and the desk average was 1 CFU / g. The number of bacteria detected in the washed candies was 4 CFU / g outdoor, 1 CFU / g in indoor and 0 CFU / g on the desk. The number of bacteria detected in non-washed chocolates was 76 CFU/g outdoor, the indoor average was 48 CFU / g, and desk average was 72 CFU / g. The number of bacteria detected in the washed chocolates was 54 CFU / g outdoor, 38 CFU / g in indoor and 57 CFU / g on a table. The number of bacteria detected in non-washed jellies was 79 CFU/g outdoor, the indoor average was 3 CFU / g, and the desk average was 69 CFU / g. The number of bacteria detected in the washing jellies was 42 CFU / g outdoor, 0 CFU / g in indoor and 39 CFU / g on a table. These results suggest that there is no harm (m = 10,000 or less, which is the value accepted by the Food Code.) Also, when the contaminated food was washed with water, the bacterial counts were significantly reduced in all three samples.

This findings are rather trivial compared with the microorganism specimens shown in Food Code, and it is confirmed that while the contamination occurs, it is insignificant. Therefore, the myth of the three-second rule is true as opposed to the figures based on the Food Code. However, more attention should be paid to subjects and periods with high sensitivity to food poisoning (Koo et al., 2014; Park et al., 2006). The experimental results of the control group were negative for bacterial growth.

This study demonstrates two significant findings. First, bacterial metastasis depends on the type and texture of food. Second, bacterial metastasis can occur in contact with moist or viscous foods (Lipschutz et al., 2016). Among the places for the experiment setting, the outdoor area was the roadside with a lot of floating population and the indoor area was the building corridor. In addition, the desk was a place for general office work. Overall, the hallway was cleaner than the desk because the hallway was thoroughly disinfected. Looking at the results from each of the samples dropped in these three places, the surface of the candy was dry and the composition was hard. The bacterial contamination was up to 41 CFU/g, which was lower than the other two samples. In the case of chocolate and jelly, the surface was sticky and moist, and it can be understood that moisture promoted bacterial transformation. A maximum of 76 CFU/g of chocolate and a maximum of 79 CFU/g of jelly were observed.

Because the popular notion supports that it takes time for bacteria to transit, it is widely acceptable to consider food left on the ground for three seconds is safe. However, according to Miranda and Schaffner (2016), it can be seen that more bacterial contamination occurs with longer contact time. At the same time, some say that the transition to some food is done instantaneously in less than a second, disproving the three-second rule. Therefore, this study emphasizes the importance of hygiene in minimizing the risk of food poisoning.

3.2. Coliform group distribution status

Coliform bacteria were used as an indicator of fecal contamination in food hygiene and also as an important indicator of food hygiene (Kim, Choi, Kim, Ding, Rahman, Bahk, & Oh, 2009). In this study, no coliform group was detected in all samples. Since coliform bacteria were not infected in a short time, we conclude that coliform bacteria are less polluted than bacteria. The test results of the control group were negative.

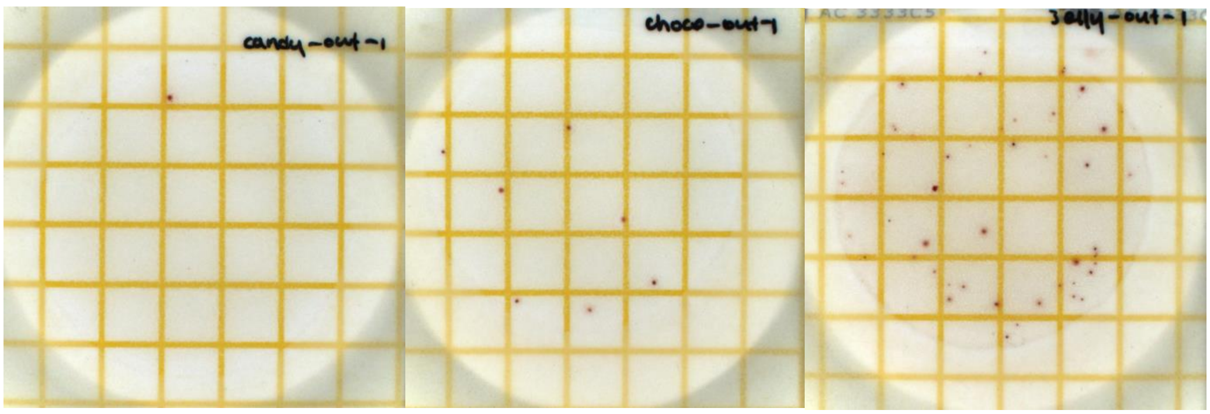

Figure 1: Colonies grown on Aerobic Count Plates.

Table 1: The result of counting general bacterial counts of each sample

Food commodity	Washing Or Non-washing	Number of bacteria (CFU/g)			
		Control	Outside	Inside	Table
Candy	Washing	0	4	1	0
	Non-washing	0	41	1	1
Chocolate	Washing	0	54	38	57
	Non-washing	0	76	48	72
Jelly	Washing	0	42	0	39
	Non-washing	0	79	3	69

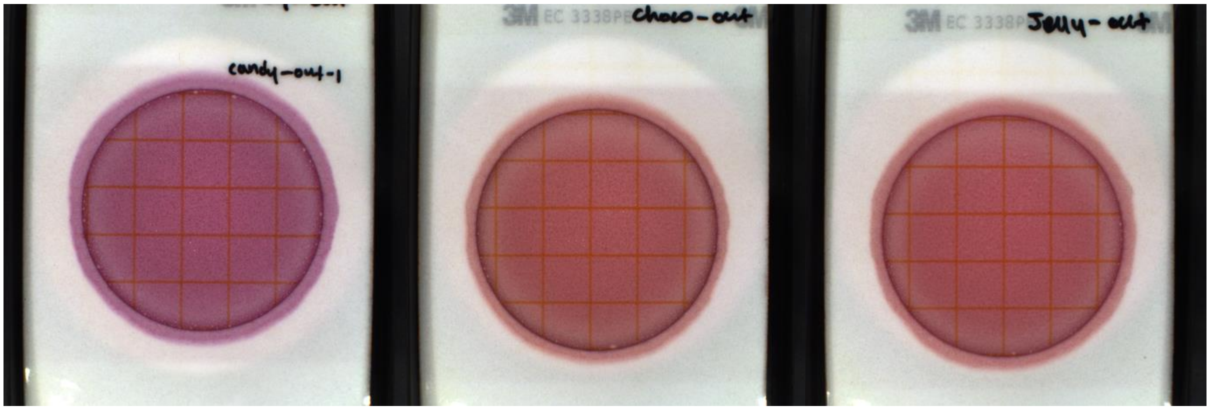

Figure 2: Colonies grown on *E.coli* / Coliform Count Plates.

4. Conclusion

In this study, Standard plate count and coliform count, which are hygienic indicator microorganisms, were tested for candy, chocolate, and jelly which are easily available and enjoyed around. After dropping each sample on the desk, indoors, and outdoors, it was immediately collected, or washed and collected to test the myth of the three-second rule. Immediately after removing the wrapping paper, each sample was dropped on the desk, indoors, and outdoors, and after three seconds from the moment of contact with the surface, and it was collected in a sample bag using sterilized sanitary gloves. After each operation, each sample was rinsed for five seconds using sterilized sanitary gloves and sterilized distilled water, and then collected in a sample bag. The number of bacteria detected in non-washed candies was 41 CFU/g outdoor and the number of bacteria detected in non-washed chocolate was 76 CFU/g outdoor. The number of bacteria detected in non-washed jellies was 79 CFU/g outdoor. Coliform group was not detected in all samples. This showed good results at the level of m = 10,000 or less, which is the value accepted by the Food Code. Also, the effect of washing the contaminated food was confirmed. This result is remarkably trivial compared with the microorganism specimens shown in Food Code, and it is confirmed that contamination occurs but not at a concerning level. Therefore, the myth of the three-second rule is true compared to the figures based on Food Code. However, it showed the characteristics of bacteria that could survive and cross-contaminate on dry food surfaces and emphasized the importance of hygiene through food contact to unsanitary surfaces to minimize the risk of food poisoning.

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