Microbial Contamination of Fruit and Vegetables and Their Disinfection

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We evaluated the microbial contamination of 17 types of vegetable and 10 types of fruit after 30-s washing with tap water with and without subsequent disinfection by 10-min immersion in 0.01% (100 ppm) sodium hypochlorite. The mean microbial contamination level of 9 types of leafy vegetable was 2.8×10^5 colony-forming units (CFU)/g after washing with water and 3.4×10^4 CFU/g after washing followed by disinfection. The mean microbial contamination level of 8 types of nonleafy vegetable was 3.4×10^4 CFU/g after washing with water and 1.0×10^4 CFU/g after washing followed by disinfection. The mean microbial contamination level of 10 types of unpeeled fleshy fruit was 9.3×10^3 CFU/g after washing with water and 1.3×10^3 CFU/g after washing followed by disinfection. The contaminants in vegetables and unpeeled fruit were similar after washing and after washing followed by disinfection, including *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. The contamination did not markedly decrease even after disinfection with sodium hypochlorite. However, the flesh of each type of peeled fruit showed no or only low levels of contamination ($\leq$10 CFU/g), probably caused by the transfer of microorganisms from the skin of fruit *via* fruit knives.

Key words vegetable; fruit; microbial contamination; *Pseudomonas aeruginosa*; disinfection

A previous study recommended "Please do not eat salads" as a note for immunocompromised patients, and the contamination of raw vegetables by microorganisms such as *Pseudomonas aeruginosa* has long been known.[1—5] Therefore, the consumption of canned fruit and cooked vegetables has been promoted among immunocompromised patients. However, many immunocompromised patients prefer to eat raw fruit and vegetables, which may also be nutritionally desirable.

The cultivation methods of vegetables and fruit have changed markedly; the main vegetable cultivation method has shifted from open-field to greenhouse cultivation. However, there have been no recent studies on the microbial contamination of fruit and vegetables. In addition, the types of fruit and vegetable which are relatively safe microbiologically for immunocompromised patients have not been fully clarified. Therefore we evaluated the microbial contamination of fruit and vegetables and the effects of sodium hypochlorite disinfection on it.

MATERIALS AND METHODS

Fruit and Vegetables We examined 17 types of vegetable [cabbage, lettuce, sunny lettuce, leek, perilla, spinach, komatsuna (Japanese mustard spinach), napa cabbage, parsley, tomato, minitomato, eggplant, cucumber, carrot, sweet pepper, onion, and Japanese radish] and 10 types of fruit (apple, pear, persimmon, grape, mandarin orange, orange, grapefruit, lemon, kiwi, and banana) served in meals in a university hospital (736 beds) between September 20 and December 20, 2006. Investigation of the microbial contamination of these fruit and vegetables was performed 3—5 times at intervals of 14 d or longer. Among the vegetables evaluated, peeled samples were used only for onion. Among fruit, unpeeled samples were used for grape and lemon, and both peeled and unpeeled samples were used for the other 8 types of fruit. The cores of apples and pears and calyces of persimmons were also evaluated.

Washing and Disinfection Fruit and vegetables were washed without scrubbing under flowing tap water (flow rate, about 7 l/min) for 30 s. Disinfection was performed by 10-min immersion in 0.01% (100 ppm) sodium hypochlorite prepared by dilution of Milton (Kyorin Pharmaceutical Co., Tokyo, Japan) with tap water. These procedures were performed while wearing sterilized rubber gloves. During immersion in sodium hypochlorite, a lid was placed directly on the samples to ensure adequate contact with the disinfecting agent.

Microbiological Analysis After washing alone or washing followed by disinfection, each sample was cut (5—20 g) using a knife wiped with ethanol for disinfection and placed in bottles containing 30 ml of sterile solution (25 ml of physiologic saline+5 ml of broth). The bottles were then vibrated with a recipro shaker (SR-1, Taiyo Industry Ltd., Tokyo, Japan) at a frequency of 250 cycles/min and an amplitude of 50 mm and ultrasonicated (Sine Sonic 100, Ikemoto Rikagaku Co., Tokyo, Japan) at 36 kHz for 5 min. Each sample was diluted 10-fold, 100-fold, and 1000-fold in sterile saline; four aliquots (0.5 ml each) of each dilution were plated on trypticase soy agar plates (Eiken Chemical Co., Tokyo, Japan), NAC agar plates (Nikken Seibutsu Co., Tokyo, Japan), salt egg yolk agar plates (Nissui Pharmaceutical Co., Tokyo, Japan), or Sabouraud dextrose agar plates (Nikken Seibutsu). Trypticase soy agar was used for the detection of general microorganisms, NAC agar for *P. aeruginosa*, salt egg yolk agar for *Staphylococcus aureus*, and Sabouraud dextrose agar for fungi. The plates were streaked with a glass "hockey stick," and trypticase soy agar and NAC agar plates were cultured at 30 °C for 24—72 h, salt egg yolk agar plates at 35 °C for 48 h, and Sabouraud dextrose agar platesat 25 °C for 2—7 d. The broth solution (5 ml) in the above sterile solution (30 ml) was used to inactivate sodium hypochlorite.[6]

Colonies were counted on each plate to determine colony-forming units (CFU), and three colonies among those on each plate were identified by Gram staining, morphologic examination, the oxidation-fermentation test, cytochrome-oxi-

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Table 1. Culture Results from Vegetables

Vegetable	No. of samples	Microbial contamination [mean (range) CFU/g]		Contaminant
		After washing with water[a)]	After washing+disinfection*[,b)]	
Cabbage	5	1.3×10^5 (6.1×10^3—4.7×10^5)	7.8×10^3 (1.2×10^2—3.2×10^4)	*Pseudomonas fluorescens*, *Sphingobacterium spiritivorum*, *Pantoea agglomerans*
Lettuce	4	3.4×10^5 (3.2×10^2—9.0×10^5)	1.1×10^5 (1.0×10^2—4.0×10^5)	*Pseudomonas fluorescens*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*
Sunny lettuce	3	7.2×10^4 (5.9×10^3—1.3×10^5)	6.4×10^3 (49—1.8×10^4)	*Pseudomonas fluorescens*, *Pseudomonas putida*, GNGB[c)]
Leek	5	1.9×10^5 (2.4×10^2—9.2×10^5)	5.2×10^4 (47—2.6×10^5)	*Acinetobacter baumannii*, *Pantoea agglomerans*, *Bacillus* spp.
Perilla	3	1.8×10^5 (1.1×10^5—2.3×10^5)	5.3×10^3 (62—1.1×10^4)	*Flavimonas orizihabitans*, *Paenibacillus alvei*, *Vibrio parahaemolyticus*
Spinach	4	2.2×10^4 (8.0×10^3—5.6×10^4)	7.0×10^3 (5.9×10^2—2.3×10^4)	*Pseudomonas aeruginosa*, *Pantoea agglomerans*, *Bacillus* spp.
Komatsuna	3	4.8×10^4 (1.8×10^4—8.3×10^4)	7.4×10^3 (1.4×10^3—1.6×10^4)	*Pseudomonas putida*, *Cryseobacterium meningosepticum*
Napa cabbage	3	2.3×10^5 (1.6×10^5—3.4×10^5)	2.1×10^3 (1.2×10^3—3.3×10^3)	*Pseudomonas fluorescens*, *Comamonas acidovorans*, *Cryseobacterium indologenes*
Parsley	4	1.3×10^6 (4.2×10^3—2.5×10^6)	1.1×10^5 (1.0×10^2—4.0×10^5)	*Pseudomonas fluorescens*, *Bacillus* spp., *Cryptococcus neoformans*
Tomato	3	4.6×10^4 (1.3×10^3—1.3×10^5)	4.0×10^3 (32—1.1×10^4)	*Pseudomonas aeruginosa*, *Pantoea agglomerans*, *Klebsiella ozanae*, *Bacillus* spp.
Minitomato	3	3.9×10^4 (2.1×10^3—8.0×10^4)	3.7×10^4 (9.7×10^2—5.9×10^4)	*Pseudomonas aeruginosa*, *Pseudomonas putida*, *Flavimonas orizihabitans*
Eggplant	3	5.4×10^2 (41—1.3×10^3)	69.1 (3—1.8×10^2)	*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Serratia phymuthica*
Cucumber	6	7.8×10^4 (5.1×10^3—1.8×10^5)	3.9×10^4 (1.0×10^3—9.3×10^4)	*Pseudomonas fluorescens*, *Ralstonia pickettii*, *Sphingomonas paucimobilis*, *Bacillus* spp.
Carrot	6	1.6×10^3 (77—8.1×10^3)	2.0×10^2 (11—6.5×10^2)	*Pseudomonas fluorescens*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, GNGB
Sweet pepper	3	1.1×10^5 (31—3.4×10^5)	7.1×10^2 (6—1.8×10^3)	*Sphingomonas paucimobilis*, *Klebsiella ozanae*
Onion[d)]	3	3.8×10^2 (2.2×10^2—6.4×10^2)	40.3 (19—64)	*Burkholderia cepacia*, *Moraxella* spp.
Japanese radish	3	1.7×10^2 (25—4.5×10^2)	1.9×10^2 (19—5.3×10^2)	*Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Brevundimonas vesicularis*

* $p<0.05$. *a*) Washing with tap water at a flow rate of 7 l/min for 30 s. *b*) Immersion in 0.01% (100 ppm) sodium hypochlorite for 10 min. *c*) Glucose nonfermentative Gram-negative bacilli that could not be identified. *d*) Flesh after peeling was evaluated.

dase test, and API system (Analytab Products, Plainview, NY, U.S.A.).

Statistical Analysis The viable cell counts were compared between washing alone and washing followed by disinfection using the Wilcoxon signed-rank test for the leafy vegetables, nonleafy vegetables, and flesh of unpeeled fruit.

RESULTS

Table 1 shows the microbial counts and predominant microbial species in vegetables after washing alone or washing followed by disinfection by 10-min immersion in 0.01% (100 ppm) sodium hypochlorite. The mean (range) of microbial counts of the nine types of leafy vegetable (cabbage, lettuce, sunny lettuce, leek, perilla, spinach, komatsuna, napa cabbage, and parsley) was 2.8×10^5 CFU/g (2.4×10^2—2.5×10^6 CFU/g) after washing and 3.4×10^4 CFU/g (47—4.0×10^5 CFU/g) after washing followed by disinfection. The mean (range) of microbial counts of the eight types of nonleafy vegetable (tomato, minitomato, eggplant, cucumber, carrot, sweet pepper, onion, and Japanese radish) was 3.4×10^4 CFU/g (25—3.4×10^5 CFU/g) after washing and 1.0×10^4 CFU/g (3—9.3×10^4 CFU/g) after washing followed by disinfection. The major contaminants were pseudomonads such as *Pseudomonas fluorescens*, *Pseudomonas putida*, and *P. aeruginosa*; *Pantoea agglomerans*; and *Bacillus* spp. *P. aeruginosa* was detected in a total of 4 samples of 4 types of vegetable among the 17 types examined. In Japanese radish, the mean number of colony-forming units per gram was enhanced by disinfection after washing. This may be due to differences in the portion of the samples examined. Among the 17 types of vegetable in Table 1, the microbial counts found in cabbage are shown in detail in Table 2. Table 3 shows the status of *P. aeruginosa* contamination in 4 samples. *P. aeruginosa* did not disappear even after disinfection in 3 of the 4 samples. In Table 3, the total number of colony-forming units per gram in tomato was enhanced by disinfection after washing. This may also be due to differences in the portion of the samples examined. *Staphylococcus aureus* was not detected in any of the 17 types of vegetable.

Table 4 shows the microbial counts and predominant microbial species after washing followed by 10-min immersion in 0.01% (100 ppm) sodium hypochlorite in the 10 types of unpeeled fruit flesh (apple, pear, persimmon, grape, mandarin orange, orange, grapefruit, lemon, kiwi, and banana). The mean (range) of microbial counts was 9.3×10^3 CFU/g (0—1.0×10^5 CFU/g) after washing and 1.3×10^3 CFU/g (0—2.5×10^4 CFU/g) after washing followed by disinfection. The contamination level was low (0—16 CFU/g) in grapefruit and persimmons. The contaminants were glucose-nonfermentative Gram-negative bacilli such as *Burkholderia cepacia* and *Brevundimonas vesicularis*, *Bacillus* spp., and yeasts such as *Candida inconspicua* and *Candida guilliermondii*. Neither *P. aeruginosa* nor *S. aureus* was detected in any of the 10 types of unpeeled fruit flesh. In pears, the total number of colony-forming units per gram was enhanced by disinfection after washing. This may be due to differences in the portion of the samples examined.

Table 2. Culture Results from Cabbage

Sample no.	CFU/g	
	After washing with water[a]	After washing+disinfection[b]
1	1.2×10^4	4.4×10^2
2	9.0×10^3	1.2×10^2
3	4.7×10^5	3.2×10^2
4	6.1×10^3	2.9×10^2
5	1.8×10^5	6.2×10^3

a) Washing with tap water at a flow rate of 7 l/min for 30 s. *b*) Immersion in 0.01% (100 ppm) sodium hypochlorite for 10 min.

Table 5 shows microbial counts and predominant microbial species in the cores of apples and pears and the calyces of persimmons. The apple cores and persimmon calyces contained 10^2—10^5 CFU/g of contaminants. Although not shown in the table, for each of the 8 fruit types after excluding grapes and lemons, 5 samples of the flesh of peeled fruit were also evaluated, but all of these samples showed no or only low levels of contamination ($\leq$10 CFU/g), probably caused by the transfer of microorganisms from the skin of the fruit *via* the fruit knife used for cutting the samples.

Wilcoxon's signed-rank test showed a significantly lower contamination level after washing followed by disinfection with sodium hypochlorite than after washing alone ($p<0.05$) for leafy vegetables, nonleafy vegetables, and unpeeled fruit.

DISCUSSION

In immunocompromised patients such as those after un-

Table 3. Detection of *Pseudomonas aeruginosa* from Vegetables

Type	No. of samples contaminated with *P. aeruginosa*/ No. of examined sample	Total CFU/g in samples with *P. aeruginosa*		CFU/g of *P. aeruginosa* in samples with *P. aeruginosa*	
		After washing with water[a]	After washing +disinfection[b]	After washing with water[a]	After washing +disinfection[b]
Spinach	1/4	1.3×10^4	5.9×10^2	1.3×10^2	0
Tomato	1/3	9.5×10^4	8.7×10^5	5.7×10^2	83
Minitomato	1/3	1.6×10^5	5.0×10^4	2.0×10^2	11
Eggplant	1/3	1.3×10^3	1.8×10^2	36	9

a) Washing with tap water at a flow rate of 7 l/min for 30 s. *b*) Immersion in 0.01% (100 ppm) sodium hypochlorite for 10 min.

Table 4. Culture Results from the Flesh of Unpeeled Fruit

Type	No. of samples	Microbial contamination [mean (range) CFU/g]		Contaminant
		After washing with water[a]	After washing+disinfection*[,b]	
Apple	7	8.7×10^2 (45—2.5×10^3)	33 (0—31)	*Brevundimonas vesicularis*, GNGB,[c] *Bacillus* spp., *Cryptococcus laerentii*
Pear	4	1.8×10^3 (0—6.4×10^3)	3.3×10^3 (0—1.2×10^4)	*Brevundimonas vesicularis*, *Burkholderia cepacia*, *Candida guilliermondii*
Persimmon	3	5.8 (0—9)	3.8 (0—16)	GNGB
Grape	4	2.6×10^4 (61—1.0×10^5)	4.2×10^2 (6—1.5×10^3)	*Pseudomonas fluorescens*, *Brevundimonas vesicularis*, *Staphylococcus auriclavis*
Mandarin orange	3	6.1×10^3 (69—1.7×10^4)	1.0×10^2 (0—2.6×10^2)	Fungi
Orange	3	5.6×10^2 (0—1.7×10^3)	1.4×10^2 (0—4.3×10^2)	*Candida lipolytica*, *Candida inconspicua*
Grapefruit	3	3.0 (0—9)	0	*Sphingomonas paucimobilis*
Lemon	3	3.1×10^4 (1.7×10^2—6.5×10^4)	3.6 (0—11)	*Candida inconspicua*
Kiwi	3	1.0×10^4 (5.8×10^2—2.5×10^4)	8.8×10^3 (6.1×10^2—2.5×10^4)	*Sphingomonas paucimobilis*, *Pantoea agglomerans*, *Bacillus* spp.
Banana	3	2.7×10^2 (14—6.6×10^2)	57 (0—1.7×10^2)	*Bacillus* spp.

* $p<0.05$. *a*) Washing with tap water at a flow rate of 7 l/min for 30 s. *b*) Immersion in 0.01% (100 ppm) sodium hypochlorite for 10 min. *c*) Glucose nonfermentative Gram-negative bacilli that could not be identified.

Table 5. Culture Results from Cores or Calyces of Fruit

Type	No. of samples	Mean (range) of CFU/g after washing+disinfection[a]	Contaminant
Apple (core)	3	1.5×10^4 (7.6×10^3—2.8×10^4)	GNGB,[b] *Bacillus* spp.
Pear (core)	4	2.3×10^2 (0—4.2×10^2)	*Pseudomonas fluorescens*, *Burkholderia cepacia*, *Cryseobacterium meningosepticum*
Persimmon (calyces)	3	2.5×10^5 (6.4×10^2—4.2×10^5)	*Candida guilliermondii*, *Pantoea agglomerans*, *Klebsiella ozaenae*

a) Washing with tap water at a flow rate of 7 l/min for 30 s followed by immersion in 0.01% (100 ppm) sodium hypochlorite for 10 min. *b*) Glucose nonfermentative Gram-negative bacilli that could not be identified.

dergoing hemopoietic cell transplantation, the oral intake of Gram-negative bacilli such as *P. aeruginosa* puts them at risk of these bacilli colonizing the intestine and subsequent infection.[7—13] Therefore, the consumption of food contaminated by Gram-negative bacilli such as *P. aeruginosa* should be minimized in immunocompromised patients.

In this study, we investigated the microbial contamination of fruit and vegetables commonly served in hospital meals in Japan, and the mean contamination level in leafy vegetables was 10^5 CFU/g, while that in nonleafy vegetables was 10^4 CFU/g after washing with water alone. Some vegetables were contaminated by *P. aeruginosa*, which is a serious pathogen in immunocompromised patients. These results were consistent with those in previous studies and support the warning that immunocompromised patients should not eat salads.[1—5]

Microbial contamination decreased significantly ($p<0.05$) after the vegetables were washed with water followed by disinfection with sodium hypochlorite compared with washing with water alone. However, it was noteworthy that the mean microbial contamination level of vegetables was 10^4 CFU/g even after disinfection with sodium hypochlorite. Therefore vegetables should be cooked before consumption by immunocompromised patients. Microorganisms in the skin of fruit and vegetables were not completely eradicated even after disinfection with sodium hypochlorite. Thus, grapes and lemons, from which it is difficult to remove the peel alone, are not appropriate for consumption by immunocompromised patients. Since the flesh of each type of peeled fruit after washing with water plus disinfection showed no or only negligible contamination, the fleshy part can be served to immunocompromised patients. When apples, pears, or persimmons are sliced, the knife should not come into contact with the cores of apples and pears or the calyces of persimmons. Among the 10 types of fruit investigated, bananas may be the most appropriate for immunocompromised patients because their peels can be removed cleanly and completely. Microbial contamination of the unpeeled flesh of grapefruit and persimmons was negligible, but the reason for this was unclear.

REFERENCES

1) Remington J. S., Schimpff S. C., *N. Engl. J. Med.*, **304**, 433—435 (1981).
2) Correa C. M., Tibana A., Gontijo Filho P. P., *J. Hosp. Infect.*, **18**, 301—306 (1991).
3) Houang E., Bodnaruk P., Ahmet Z., *J. Hosp. Infect.*, **17**, 125—131 (1991).
4) Khan M. R., Shaha M. L., Kibria A. H. M. G., *Microbiology*, **14**, 88—90 (1992).
5) Nascimento M. S., Silva N., Catanozi M. P., Silva K. C., *J. Food Prot.*, **66**, 1697—1700 (2003).
6) Kelsey J. C., Mackinnon I. H., Maurer I. M., *J. Clin. Pathol.*, **27**, 632—638 (1974).
7) Bert F., Maubec E., Bruneau B., Berry P., Lambert-Zechovsky N., *J. Hosp. Infect.*, **39**, 53—62 (1998).
8) File T. M., Tan J. S., Thomson R. B., Stephens C., Thompson P., *Infect. Control Hosp. Epidemiol.*, **16**, 417—418 (1995).
9) Horcajada J. P., Martinez J. A., Alcon A., Marco F., Lazzari D. E., Matos A., Zaragoza M., Salles M., Zavala E., Mensa J., *Infect. Control Hosp. Epidemiol.*, **27**, 774—777 (2006).
10) Levy J., Van L. Y., Verhaegen G., Perpete C., Butzler J. P., Wenzel R. P., *J. Parenter. Enteral Nutr.*, **13**, 228—234 (1989).
11) Rossolini G. M., Mantengoli E., *Clin. Microbiol. Infect.*, **11**, 17—32 (2005).
12) Thuong M., Arvaniti K., Ruimy R., De la Salmoniere P., Scanvic-Hameg A., Lucet J. C., Regnier B., *J. Hosp. Infect.*, **53**, 274—282 (2003).
13) Thurn J., Crossley K., Gerdts A., Maki M., Johnson J., *J. Hosp. Infect.*, **15**, 203—217 (1990).