Bacterial Contamination of Vegetables Served in Hospitals

Chertsak Dhiraputra MD *, Chuntima Tiensasitorn MSc*,
Wanida Techachaiwiwat MSc*, Naruemol Jirapanakorn MSc*,
Kanchana Kachintorn RN**, Somwang Danchaivijitr MD***

**Department of Microbiology, **Center for Nosocomial Infection Control, ***Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok*

Objectives : *To study bacterial contamination of fresh vegetables before cleaning and before serving to patients in 14 hospitals.*

Material and Method : *Aerobic plate count was performed and emphasized on total viable aerobic bacteria, fecal coliform, fecal Escherichia coli and enteric pathogens in fresh vegetables including romaine lettuce, onion, parsley, celery and tomato before cleaning and before serving. Hospital nutrition officers who were involved in food purchasing and processing were interviewed.*

Results : *One hundred and six of 403 of fresh vegetable samples (26.3%) before cleaning were contaminated with > 10^7 colony forming unit per gram (CFU/gram) of viable aerobic bacteria, 106 of 178 samples (59.6%) contained MPN/fecal coliform >1,100 / gram, 78 samples (43.8%) contained MPN fecal E.coli >10/gram. Enteric bacteria were isolated from 7.2% of the total 304 samples including non typhoid Salmonella (1 sample), Vibrio cholerae non O1/O139 (7 samples) and Aeromonas species (14 samples). Forty of 396 ready to serve vegetable samples (10.1%) contained > 10^7 CFU/gram of viable aerobic bacteria. Seventy five of 183 (40.9%) samples contained >1,100 MPN fecal coliform/gram and 43 (23.5%) contained >10 MPN fecal E. coli/gram. Enteric bacteria were also detected in 7.6% of the samples including V. cholerae non O1/O139 (6 samples) and Aeromonas species (17 samples).*

There were three different ways in obtaining fresh vegetables to the hospitals : by auction (50%), wholesalers (21.4%) and retailers (14.2%). There were also different standards of transportation, packaging, delivery and food processing, particularly cleaning methods.

Conclusion : *Ready-to-eat fresh vegetables were contaminated in high percentages with microorganisms in the number that exceeded the standard. Better management is required to safeguard patients.*

Keywords : *Bacterial contamination, Vegetables, Hospital*

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Foodborne illness is still a major problem in many countries. The source of causative organisms include fresh fruits and vegetables. Vegetables are essential food for healthy persons and also for patients particularly, fresh vegetables. Nowadays, consumption of fresh vegetables has largely increased among Thai people. Preparation and handling ready-to-eat vegetables must be performed with care, since vegetables planted in growing fields could be contaminated with soil microbes or even with human fecal flora and enteric pathogens. Human intestinal infections such as salmonellosis, shigellosis and cholera caused by comsumption of raw vegetables have been reported in many countries.[1-4] Enteric pathogens such as *Aeromonas, Salmonella, Shigella, Escherichia coli* O157:H7, *Vibrio cholerae, Bacillus cereus* and *Campylobacter jejuni* have been isolated from a wide variety of fresh produce: lettuce, celery, sprout, green onion, broccoli, cauliflower, pepper, spinach, mush-

Correspondence to : Danchaivijitr S, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. E-mail : sisdc@mahidol.ac.th

room, tomato, cabbage, chilli, strawberries and water melon.[1] In addition, nosocomial outbreaks of *Salmonella*,[5-9] *Listeria*[10-11] and *Vibrio*[3] have been reported. In Thailand, the warm and humid temperature all year round is suitable for bacterial multiplication. Food poisoning in Thailand including nosocomial diarrhea are common. Food hygiene in hospitals must be strictly maintained since patients are more vulnerable to infections than normal persons. Contamination of pathogens to prepared foods could occur during food processing by canteen personnel. Therefore, appropriate preparation particularly, for vegetables served fresh must be implemented. Harvesting, packaging, and transportation from farms to hospitals are difficult to control, good food processing is ,thus, required to ensure clean food. Information on food microbiology, handling and processing of vegetables are needed to improve food hygiene in hospitals. The present study was to determinate the number of viable bacteria and enteric pathogens : salmonella, shigella, vibrios and aeromonas in vegetables on arrival at hospital canteens and when they are ready to be served to patients.

Material and Method

The study was done during March 2002 and January 2003. Fourteen hospitals were enrolled by randomized stratified sampling. Information on sources, providers, transportation and preparation of vegetables was done by interviewing hospital canteen personnel. Samples of romaine lettuce, onion, parsley, celery and tomato were collected before and after processing by co-operating infection control nurses (ICNs) for bacterial culture. Quantitative cultures were done for viable aerobic bacteria, fecal coliform, *Escherichia coli* and *Salmonella, Shigella, Vibrio,* and *Aeromonas* spp. Results were reported as number of bacteria per 1 gram of vegetables.

Determination of viable aerobic bacterial count and enteric pathogens in fresh vegetable

Twenty-five grams of vegetables was mixed thoroughly with 225 ml of peptone broth. The suspension was ten fold diluted and examined for aerobic plate count on enriched nutrient agar. Colony count was done after 48 hours incubation at 35^{O}C using spread plate technique as previously described[12]. Each 1 ml. of the suspension was also transferred into gram-negative broth for isolation of salmonella and shigella and into alkaline peptone for vibrios and aeromonas. Isolation and identification of the enteric pathogen were performed as previously described[13].

Determination of fecal coliform and Escherichia coli in fresh vegetable

The vegetable suspensions were used to determine the most probable number (MPN) per gram of vegetables using the multiple tube MPN technique[14].

Results

Four hundred and three of raw and prepared fresh vegetable samples and 396 ready to eat fresh (REF) vegetable samples consisted of romaine lettuce, onion, parsley, celery and tomato were examined for viable aerobic plate count and enteric pathogens such as salmonella, shigella, vibrios and aeromonas. Results of bacterial culture are shown in Tables 1-3.

The results of total viable aerobic bacterial count of vegetables from 14 hospitals are shown in Table 1. Forty six point nine per cent of the total raw samples carried viable bacteria less than 10^6 CFU/gram. Twenty six point eight per cent of the samples carried $10^{(6-7)}$ colony forming units (CFU) per gram. Twenty six point three per cent carried viable bacteria over 10^7 CFU/gram. Rates of contamination of raw vegetable over 10^7 CFU/gram were as follows; romaine lettuces 39/128 (30.5%), onion 25/77 (32.5%), parsley 15/44

Table 1. Total viable aerobic bacterial count per gram of fresh vegetables

Vegetables	Total	Raw			Total	Ready to serve		
		$<10^6$	10^6-10^7	$>10^7$(%)		$<10^6$	10^6-10^7	$>10^7$(%)
Romaine lettuce	128	39	50	39 (30.5)	127	75	32	20 (15.7)
Onion	77	32	20	25 (32.5)	72	56	13	3 (4.2)
Parsley	44	21	8	15 (34.1)	37	20	10	7 (18.9)
Celery	36	13	8	15 (41.7)	36	19	9	8 (22.2)
Tomato	118	84	22	12 (10.2)	124	110	12	2 (1.6)
Total	403	189	108	106 (26.3)	396	280	76	40 (10.1)

Satisfactory < 10^6 Acceptable 10^6 - 10^7, Unsatisfactory >10^7

Table 2. Most probable number (MPN) of fecal coliform and *Escherichia coli* count per gram of fresh vegetables

Vegetables	Total	MPN of fecal coliform			MPN of *E. coli*		
		<3	3-1,100	>1,100	<3	<10 (%)	10- >1,100 (%)
Romaine lettuce							
Raw	55	5	8	42(76.4%)	23	23(41.8)	23(41.8)
Ready to eat	56	4	23	29(51.8%)	30	38(67.9)	18(32.1)
Onion							
Raw	34	3	5	26(76.5%)	20	20(58.8)	14(41.2)
Ready to eat	35	5	14	16(45.7%)	27	29(82.9)	6(17.1)
Parsley							
Raw	17	1	6	10(58.9%)	12	12(70.6)	5(29.4)
Ready to eat	17	4	6	7(41.2%)	10	13(76.5)	4(23.5)
Celery							
Raw	19	0	7	12(63.2%)	9	9(47.4)	10(50.0)
Ready to eat	20	3	4	13(65.0%)	9	10(50.0)	10(50.0)
Tomato							
Raw	53	11	26	16(30.2%)	36	36(67.9)	17(32.1)
Ready to eat	55	12	33	10(18.9%)	46	50(90.9)	5(9.1)
Raw	178	20	52	106(59.6%)	100	100(56.2)	78(43.8)
Ready to eat	183	28	80	75(40.9%)	122	140(76.5)	43(23.5)

Total 3. Enteric pathogens isolated from the vegetables

Vegetables	**Total**	**Raw**			**Ready to serve**		
		Salmonella#	*Vibrio**	*Aeromonas*	*Salmonella#*	*Vibrio**	*Aeromonas*
Romaine lettuce	96	-	3	7	-	3	9
Onion	59	1	2	-	-	1	-
Parsley	29	-	-	2	-	-	-
Celery	25	-	2	4	-	2	4
Tomato	95	-	-	1	-	-	4
Total	304	1	7	14	-	6	17
Percentage	100	0.3	2.3	4.6	-	2.0	5.6

non-typhi *Salmonella*, * *Vibrio cholerae* non O1/O139

(34.1%), celery 15/36 (41.7%) and tomato 12/118 (10.2%). After they were cleaned and were ready to be served to patients, 40 of 396 (10.1%) of the vegetables still carried viable bacterial cells over 10^7 CFU per gram. Rate of contamination was as follows; romaine lettuce 20/127 (15.7%), onion 3/72 (4.2%), parsley 7/37 (18.9%), celery 8/36 (22.2%) and tomato 2/124 (1.6%).

Table 2 shows the results of fecal coliform and *E. coli* most probable number (MPN) per gram of the raw vegetables and cleaned vegetables. Contamination of raw vegetables by fecal coliform was found in most of the samples. Fecal coliform count of raw and cleaned vegetables over 1,100 MPN/gram, were : total vegetables 106/178 (59.6%) and 75/183 (40.9%), romaine lettuce 42/55(76.4%) and 29/56 (51.8%), onion 26/34 (76.5%) and 16/35 (45.7%), parsley 10/17 (58.9%) and 7/17 (41.2%), celery 12/19 (63.2%) and 13/20(65.0%) and tomato 16/53 (59.6%) and 10/55 (18.9%) respectively. The fecal *E.coli* count below 10 MPN/gram of raw and cleaned vegetables were : total vegetables 78/178 (43.8%) and 43/183(23.5%), romaine lettuce 32/55(58.2%) and 8/56(32.1%), onion 14/34 (41.2%) and 6/35 (17.1%), parsley 5/17 (29.4%) and 4/17 (23.5%), celery 10/19 (52.6%) and 10/20 (50.0%) and tomato 17/53 (32.1%) and 5/55 (9.1%).

The contamination of enteric pathogens from

raw vegetables and cleaned vegetables is shown in Table 3. *Salmonella* species (not *S. typhi*) was isolated from 1 sample (0.3%) of raw onion. *V. cholerae* non O1/O139 was isolated from 7 samples (2.3%) of raw vegetables and 6 samples (2.0%) from cleaned vegetables. *Aeromonas* species included *A. hydrophila* and *A. sorbia* were isolated from 14 samples (4.6%) of raw and 17 samples (5.6%) of cleaned vegetables.

Table 4 shows the supplying sources of vegetables for the 14 hospitals. Only 3 hospitals obtained the vegetables from known and regular producers. Seven hospitals (50.0%) bought the vegetables from companies. Three hospitals obtained them from wholesalers and 2 hospitals obtained them from retailers. Two hospitals bought the vegetables from more than one source.

Transportation of vegetables from providers to the hospitals is shown in Table 5. In 5 hospitals (35.7%) the vegetables were transported in closed and clean containers and to 1 hospital in temperature controlled vehicles. Transported in separate containers was reported from 8 hospitals (57.1%).

Preparation process of the vegetables is shown in Table 6. In 8 hospitals (57.1%) they were processed in separate rooms and in 13 (92.9%) with separate sets of cooking tools. Preparation of the vegetables was done by trained staff in 11 hospitals (78.6%). In 8 hospitals (57.1%) the raw vegetables were treated with potassium permanganate or diluted calcium chloride solution. In 3 hospitals (21.4%) the cleaned vegetable were kept in close containers. All hospitals used closed food delivery carts in transporting food to patients. Freshly prepared vegetables were served to patients within 2 hours in 11 hospitals (78.6%). While waiting for food delivery to wards, the vegetables were kept in refrigerators in 3 out of 6 hospitals reported.

Discussion

Gastrointestinal infection is a very common health problem in developing countries. Patients with low immunity have a high risk of exposure to pathogenic bacteria in food. The infection in immunocompromized patients is more severe and could lead to death.

Standards of safety food have been set[15,16]. Acceptable microbiological guidelines for ready-to-eat fresh vegetables include total aerobic count of less than 10^7 CFU per gram, MPN of fecal *E. coli* less than 10 CFU per gram and undetectable amount of enteric pathogens including *Salmonella, E. coli* O157:H7 and *V. cholerae* in 25 grams of fresh vegetables. Foods served to patients should strictly follow the standards.

The results in the present study showed that the average aerobic bacterial plate count of raw vegetables exceeding the acceptable level (>10^7CFU/gm) was 26.3%. The result was similar to a previous study.[17] Even after processing the contamination remained as high as 10%. High levels of fecal coliform (MPN >1,100/

Table 4. Supplying sources of vegetables

Sources	Number of hospitals	Per cent
Producer		
Known and regular growing field	3/14	21.4
Providers		
Companies	7/14	50.0
Wholesalers	3/14	21.4
Retailers	2/14	14.3
Combination	2/14	14.3

Table 5. Transportation of vegetables to the hospitals

Transportation	Number of hospitals	%
In temperature controlled vehicles	1/14	7.1
In closed containers	5/14	35.7
In separate containers	8/14	57.1

Table 6. Preparation and transportation of the vegetables in hospitals

Preparation/Transportation	Number of hospitals	%
In separate room	8/14	57.1
Using separated cooking tools	13/14	92.9
By trained personnel	11/14	78.6
Using disinfectants	8/14	57.1
Using cleaned water	6/14	42.9
Keeping cleaned vegetables in clean and closed containers	3/14	21.4
Keeping in refrigerators before serving	3/6	50.0
Transporting prepared food in closed carts	14/14	100.0
Serving within 2 hours after preparation	11/12	91.7

gm) and fecal *E. coli* (MPN >10/gm) were found in 59.6% and 43.8% of raw vegetables. The contamination only slightly decreased after cleaning, indicating defective processing methods. In addition, the presence of fecal *E. coli* indicates the possibility of enteric pathogens contamination. In the present study, 7.2 % of the raw vegetables were contaminated by non typhi *Salmonella*, *V. cholerae* non O1/O139 and *Aeromonas* species while 7.6% of cleaned vegetables were still contaminated with *V. cholerae* non O1/O139 and *Aeromonas* species. The increase in contamination of the cleaned vegetables with *Aeromonas* species could occur during food processing.

Different sources of supply resulted in inappropriate methods in packaging and transportation. These practices increase the risk of contamination of vegetables.

Several investigators reported the efficacy of using chlorinated water in decontamination of raw vegetables[18-21]. In the present study, more than half of the hospitals used chlorinated or permanganate solutions. Even though the number of contaminated vegetables was reduced, 23.5% of cleaned vegetables were still contaminated with *E. coli*. Various factors including concentrations of disinfectants, duration of immersion, number of washing, etc., play an important role in decontamination. A standard protocol for decontamination of vegetables is clearly needed.

Prepared food left in room temperature in a warm climate allows the multiplication of contaminating bacteria. If the food could not be served immediately after preparation, it should be stored in a refrigerator.

Conclusion

The vegetables in the present study were contaminated with a high number of bacteria that exceeded the acceptable levels both before and after cleaning. The contaminated organisms included fecal coliforms, fecal *E. coli* and some enteric pathogens. The whole process including purchasing, transport, washing, decontaminating, storage and serving in the hospitals should be reviewed.

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References

1. Buck JW, Walcott RR, Beuchat LR. Recent trends in microbiological safety of fruits and vegetables. Plant Health Progress doi : 10.1094/php-2003-0121-01-rv. (Accessed October 5, 2005 at http://www.sproutnet.com/Research/recent trends in microbiological.htm).
2. Beuchat LR. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microb Infect 2002; 4: 413-23.
3. Rabbani GH, Greenough WB. Food as a vehicle of transmission of cholera. J Diarrhoeal Dis Res 1999; 17: 1-9.
4. Norazah A, Rahizan I, Zainuldin T, Rohani MY, Kamel AG. Enteropathogenic *Escherichia coli* in raw and cooked food. Southeast Asian J Trop Med Public Health 1998; 29: 91-3.
5. Guallar C, Ariza J, Dominguez MA, Pena C, Grau I, Verdaguer R, et al. An insidious nosocomial outbreak due to Salmonella enteritidis. Infect Control Hosp Epidemiol 2004; 25: 10-5.
6. Dryden MS, Keyworth N, Gabb R, Stein K. Asymptomatic foodhandlers as the source of nosocomial salmonellosis. J Hosp Infect 1994; 28: 195-208.
7. Hedberg CW, Angulo FJ, White KE, Langkop CW, Schell WL, Stobierski MG, et al. Outbreaks of salmonellosis associated with eating uncooked tomatoes: implication for public health. Epidemiol Infect 1999; 122: 385-93.
8. Wood RC, Hedberg C, White K. A multi-state outbreak of *Salmonella javiana* infections associated with raw tomatoes (abstract). Proceedings of the 40th Annual Conference of the Epidemic Intelligence Service. Atlanta : Centers for Disease Control and Prevention, 1991: 69.
9. Standaert SM, Hutcheson RH, Schaffner W. Nosocomial transmission of salmonella gastroenteritis to laundry workers in nursing home. Infect Control Hosp Epidemiol 1994; 15: 22-6.
10. Elsner HA, Tenschert W, Fischer L, Kaulfers PM. Nosocomial infections by Listeria monocytogenes: analysis of a cluster septicemias in immunocompromised patients. Infection 1997; 25: 135-9.
11. Graham JC, Lansert S, Bignardi G, Hollyoak V. Hospital-acquired listeriosis. J Hosp Infect 2002; 51: 136-9.
12. Garbutt J. Essentials of food microbiology. Controlling the microbiological quality and safety of foods. London: Arnold, 1997: 207-13.
13. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manuals of clinical microbiology. 7th ed. Washington DC : American Society for Microbiology, 1999: 438-516.

14. Genta ML, Heluane H. Biochemical identification of most frequently encountered bacteria that cause food spoilage. In: Spencer JFT, Ragout de Spencer AL, editors. Food microbiology protocols. New Jersey: Humana Press Inc, 2001: 18-20.
15. Standards for Food Microbiology for General Foods, Ready to Eat Foods, and Raw foods. Section of Food, Department of Medical Science, Ministry of Public Health of Thailand. Available from URL http://www.dmsc.moph.go.th/webroot/BQSF/File/varity/cheme/conflict22.htm
16. Guidelines for the interpretation of results of microbiological analysis of some ready-to-eat foods sampled at point of sale. Food Safety Authority of Ireland, Dublin: 2001; 1-12. Available from URL: http://www.fsai.ie/publications/guidance_notes/gn3.pdf
17. Viswanathan P, Kaur R. Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. Int J Hyg Environ Health 2001; 203: 205-13.
18. Wei CI, Huang TS, Kim JM, Lin WF, Tamplin ML, Baryz JA. Growth and survival of Salmonella montevideo on tomatoes and disinfection with chlorinated water. J Food Prot 1995; 58: 829-36.
19. Hollyday SL, Scouten AJ, Beuchat LR. Efficacy of chemical treatments in eliminating Salmonella and Escherichia coli O157:H7 on scarified and polished alfalfa seeds. J Food Prot 2001; 64: 1489-95.
20. Beuchat LR, Ward TE, Pettigrew CA. Comparison of chlorine and a prototype produce wash product for effectiveness in killing Salmonella and Escherichia coli O157: H7 on alfalfa seeds. J Food Prot 2001; 64: 152-8.
21. Weissinger WR, Chantarapanont W, Beuchat LR. Survival and growth of Salmonella baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. Int J Food Microbiol 2000; 62: 123-31.

การปนเปื้อนแบคทีเรียของผักสดของโรงพยาบาล

เชิดศักดิ์ ธีระบุตร, จันทิมา เทียนศศิธร, วนิดา เตชะชัยวิวัฒน์, นฤมล จิระพนาคร, กาญจนา คชินทร, สมหวัง ด่านชัยวิจิตร

วัตถุประสงค์ : *ศึกษาการปนเปื้อนแบคทีเรียในผักสดก่อนทำความสะอาดและก่อนนำส่งให้ผู้ป่วยบริโภคในโรงพยาบาล*

วัสดุและวิธีการ : *ตรวจ viable aerobic bacteria, fecal coliform, fecal Escherichia coli และ enteric pathogens ในผักสดได้แก่ ผักกาดหอม ต้นหอม ผักชี ขึ้นฉ่าย และมะเขือเทศก่อนทำความสะอาด และก่อนนำส่งให้ผู้ป่วยบริโภค วิเคราะห์ข้อมูลจากการสัมภาษณ์ผู้รับผิดชอบฝ่ายโภชนาการของโรงพยาบาล เกี่ยวกับการจัดซื้อ นำส่ง และกระบวนการเตรียมผักสดเพื่อให้ผู้ป่วยบริโภค*

ผลการศึกษา : *ผักสดก่อนทำความสะอาด 403 ตัวอย่าง มี viable aerobic bacteria > 10^7 colony forming unit/gram 26.3% 178 ตัวอย่างมี MPN fecal coliform > 1,100 /gram 59.6% และ MPN fecal Escherichia coli >10 /gram 43.8% ตรวจพบ enteric bacteria 7.2% ได้แก่ non typhoid Salmonella 1 ตัวอย่าง Vibrio cholerae non O1/O139 7 ตัวอย่าง และ Aeromonas species 14 ตัวอย่าง ผักสดหลังผ่านกระบวนการเตรียมและพร้อมส่งให้ผู้ป่วย 396 ตัวอย่าง มี viable aerobic bacteria > 10^7 colony forming unit (CFU)/gram 10.1% MPN fecal coliform / gram > 1,100 40.9% และ MPN fecal E. coli /gram >10 23.5% ตรวจพบ enteric bacteria 7.6% ได้แก่ Vibrio cholerae non O1/O139 6 ตัวอย่าง และ Aeromonas species 17 ตัวอย่าง*

โรงพยาบาลมีการจัดหาผักสดหลายรูปแบบ ได้แก่ การประมูล (50%) ผู้ขายส่ง (21.4%) ผู้ขายปลีก (14.2%) การนำส่งแตกต่างกันทั้งด้านการบรรจุ และการขนส่ง กระบวนการทำความสะอาดและทำลายเชื้อแตกต่างกัน การเตรียมผักสดเกือบทุกโรงพยาบาลใช้บุคลากรที่ผ่านการอบรมแล้ว ในห้องและใช้อุปกรณ์แยกเฉพาะ

สรุป : *ผักสดที่พร้อมให้ผู้ป่วยรับประทานมีการปนเปื้อนเกินมาตรฐานในอัตราสูง แหล่งซื้อ การนำส่ง กระบวนการในการเตรียม แตกต่างกันสำหรับในแต่ละโรงพยาบาล ควรจัดทำมาตรฐานการจัดการผักสด เพื่อสวัสดิภาพของผู้ป่วย*