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Potential source of infection through vegetables with a note on bacterial pathogens isolated from children with diarrhoea

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Distribution of bacterial pathogens was studied on 350 specimens of vegetables from bazaars and salads from street vendors in 10 townships in three seasons during March 2000 to August 2001. Similarly, 62 cases of diarrhoeic children attended the Yangon Children's Hospital during May to August, 2001 were tested for isolation of pathogens. All the vegetables and salads were heavily contaminated with coliforms and faecal coliforms. *Salmonella* species, *Shigella dysenteriae* and *S. sonnei* were isolated from Thaketa, Insein and Mingaladon respectively. *Vibrio cholerae* was isolated from Tamwe and Kamayut. *S. dysenteriae* was isolated from coriander (nan-nan-pin) and *V. cholerae* from hsala. Various serogroups of *Escherichia coli* (O6K15, O28a/cK73, O25K+, O86K62, O142K+, O146K89, O148K+) were isolated from vegetables: *Aeromonas hydrophilia*, enteropathogenic *E. coli* (O27K+, O28a/cK73, O111K58, O114K90, O119K69, O128K67, O148K+, O157K+, O159K+), *Plesiomonas shigelloides*, *S. dysenteriae* A, *S. sonnei* phase 1, *V. cholerae* O1 (Ogawa) and *V. cholerae* O139 were isolated from 27 cases (43.55%) of diarrhoeic children. Studies on antibiotic resistance and plasmid patterns reveal that some clinical and environmental *E. coli* possess similar pattern of resistance gene. Thus, environmental health care is important to eliminate transmission of infections. From experiments, just washing with clean water 11 times could not eliminate faecal coliforms totally. However, washing vegetables at least three times with clean water (1 litre each for 50 grams) and treating with 0.001 percent of potassium permanagate for 30 minutes and again washing out with clean water for three times could eliminate faecal coliforms.

INTRODUCTION

Environmental risk factors play an important role on health in many countries. Children are exposed to a range of health risks within their environment and in many cases they play, or work, in environments that are detrimental to health. Contaminated water and food increase the risk of enteric infections including cholera, shigella and salmonella. Young children are at high risk of exposure because they are usually with their mothers around the cooking area. Every year about 11 million children die before they reach their fifth birthday. Of these, about 8 million children die from no more than five infections including diarrhoeal diseases [1]. As World Health Organization has estimated that up to 70 percent of diarrhoeal diseases may be due to contaminated food and water, these vehicles may be responsible for the majority of the reported illness annually and a great number more unreported [2]. This research, thus, aims to find out transmission of enteric pathogens through vegetables, pasteurized Yangon Children's Hospital during 2000 to milk and diarrhoeic children attended 2001.

MATERIALS AND METHODS

Study period

March 2000 to September 2001.

Transportation

Vegetables in plastic bags, milk as it is cooled and rectal swabs from children in Cary Blair medium through cold chain were transported within 30 minutes to three hours from collected area to the laboratary.

Sample size

A total of 320 specimens of vegetables, (gazun, kyethunmeik, khayanthi, gobidouk, nannanpin,pinzein, pusinan, mounlaouhpyu, mounlapin, myinkhwa, shaukywet, thayetkin and thakhwathi) from 10 township bazaars (Pabedan, Ahlone, Insein, Tamwe, Mingala- don, S. Okkalapa, Kamayut Thaketa, Sanchaung and Yankin) were tested in three seasson. Also, salads (tosaya) from 30 street vendors (htaminsaing) which were located in those townships were included in this study. At least five to 10 kinds of varieties were prepared as tosaya either in fresh, boiled or pickled forms in each preparation. Also, 62 cases of diarrhoeic children attended at Yangon Childrens Hospital were studied for isolation of enteric pathogens.

Determination of coliforms and faecal coliforms

Presumptive test by multiple tube technique was done by using MacConkey Broth Purple using single and double strength and the presence of coliforms and faecal coliforms was determined by incubating at 37˚C and 44˚C respectively. Confirmation was done by using Brilliant Green Bile medium with their corresponding presumptive positive tube using respective temperatures [3].

Determination of Total Bacterial Count

Dilutions were done by sterile phosphate buffer if necessary and 0.02 ml of each dilutions was placed onto Nutrient agar plates, allowed to dry at room temperature and incubated at 37˚C [4].

Determination of enteric bacterial pathogens

Approximately 20 ml of samples were spinned and the residue was used for inoculation onto Blood agar, MacConkey agar, Salmonella-Shigella agar, Mannitol Salt agar, Thiosulphate Citrate Bile Sucrose agar for primary isolation. Simultaneously, enrich-ment media of Selenite F and Alkaline Peptone water were used for secondary inoculation. The suspected colonies were then picked from respective media and were confirmed biochemically and serologically [5].

RESULTS

Environmental studies from vegetables in different townships

As shown in Table 1, all the tested vegetables in three seasons from 10 townships were contaminated with coliforms and faecal coliforms. *Shigella dysenteriae* and *S. sonnei* were isolated from Insein and Mingaladon respectively in October, whereas, *Vibrio cholerae* was isolated from Tamwe in August and Kamayut in November respectively. Moreover, *Salmonella* species was isolated from Thaketa in August and October.

Isolation of bacterial pathogens from children attended the Yangon Children's Hospital during 2001

As shown in Table 2, bacterial pathogens were isolated from 27 cases. These include *Aeromonas hydrophila* (3.23%), *Escheri-chia coli* (EPEC) (19.36%), *Plesiomonas shigelloides* (1.61%), *Shigella dysenteriae* A (3.23%), *Shigella sonnei* phase1(4.84%), *Vibrio cholerae* O1 (8.07%), and *Vibrio cholerae* O139 (3.23%). *A. hydrophila, P. shigelloides, S. sonnei* and *V.cholerae* O1 were isolated from 4 cases (44.44%) of the age group of <6 months. From age group of 6-12 months, S. *dysenteriae, S. sonnei, V. cholerae* O139, *E.coli* EPEC (O28acK73,

O114K90, O128K67 & O159K+) were isolated from 9 cases (45%). From age group of 13-24 months, *A.hydrophila,E. coli* (O27K+, O119K69, O148K+, O157K+ &O159K+) were isolated. From the age groupof 25-36 months, *S. dysenteriae, S. sonnei* and *V. cholerae* O1 were isolated. *V. cholerae* was isolated from age group of 37-48 and 48-60 months (Table 3).

Association of bacterial pathogens isolated from stool samples and from vegetables in different townships

As shown in Table 4, it was noted that isolation of same species had occurred in Insein and Mingaladon with *S. dysenteriae* and *S. sonnei* phase 1 from stool samples and vegetables respectively.

Total bacterial count in different types of vegetables after washing

A total of 14 different kinds of vegetables weighing 2.2 to 140.4 grams in each case due to the availability of the products were washed with two litres of clean water (free from coliforms and faecal coliforms) up to eleven times. Total bacterial count, coliform and faecal coliform counts were performed by Miles and Misra method. The count obtained after washing is shown in Table 5.

Table 1. Bacterial pathogens isolated from vegetables in differenet places in three seasons

Place	Hot & Hot wet 7-3-00 to 11-7-00			Wet 18-7-00 to 16-9-00			Cool 21-9-00 to 4-12-00		
Papedan	1	7-3-00	C +FC	12	18-7-00	O119K69 C +FC	22	21-9-00	O28a/c C +FC
Ahlone	2	13-3-00	C +FC	13	25-7-00	C +FC	23	2-10-00	O25K+ O86K62 O142K+ O148K+ C +FC
Insein	3	21-3-00	O126K71 C +FC	14	31-7-00	O28a/c O111K58 C +FC	24	9-10-00	*S. dys.* C +FC
Tamwe	5	2-5-00	C +FC	15	8-8-00	O28a/c O111K58 O125K+ *V. c.*(O) C +FC	28	13-11-00	C +FC
Mingaladon	6	31-5-00	O126K71 C +FC	16	15-8-00	O86K61 O111K58 O142K+ C +FC	25	16-10-00	*S. son.* C +FC
S.Okkalapa	7	6-6-00	C +FC	17	21-8-00	C +FC	26	23-10-00	C +FC
Kamayut	8	13-6-00	C +FC	19	3-9-00	O111K58 O119K69 C +FC	29	21-11-00	O146K89 *V. c.*(O) C +FC
Thaketa	9	20-6-00	O26K60 O86K62 C +FC	18	28-8-00	O111K58 O142K+ O148K+ *Salmo.* C +FC	27	30-10-00	O6K15 O146K69 *Salmo.* C +FC
Sanchaung	10	4-7-00	C +FC	20	11-9-00	O111K58 O28a/c C +FC	30	27-11-00	*S. dys.* C +FC
Yankin	11	11-7-00	C +FC	21	16-9-00	O28a/c C +FC	31	4-12-00	C +FC

C = Coliforms serogroups FC = Faecal coliforms EPEC = Enteropathogenic *Escherichia coli*

S. dys.= *Shigella dysenteriae* *S. son.* = *Shigella sonnei*

Salmo. = *Salmonella* species *V. c.*(O) = *Vibrio cholerae* (Ogawa)

Table 2. Bacterial pathogens isolated from children with diarrhoea (62 cases) attended the Yangon Children's Hospital during June-August 2001

Pathogens isolated	Males n=34	%	Females n=28	%	Total
A. hydrophila	2	5.88	0	0	2 (3.23)
E. coli (EPEC)	10	29.41	2	8.00	12 (19.36)
P. shigelloides	1	2.94	0	0	1 (1.61)
S. dysenteriae A	1	2.94	1	3.57	2 (3.23)
S. sonnei phase 1	2	5.88	1	3.57	3 (4.84)
V. cholerae O1	3	8.82	2	7.14	5 (8.07)
V. O139	1	2.94	1	3.57	2 (3.23)
Total	20	58.82	7	25.00	27 (43.55)

Figures in parenthesis denote percentages

It was found that the count decreased to 36 organisms/ml in lemon leaves after washing four times. The radish plants which were smeared with soil in roots still contained uncountable number of bacteria even after washing up to 11 times. However, these bacteria were not detected after dipping the vegetables in 0.001% potassium permanganate for 30 minutes and washed again three times with clean water.

DISCUSSION

A food-borne disease outbreak (FBDO) is defined as an incident in which two or more persons experience a similar illness resulting
from the ingestion of a common food. In Myanmar, diarrhoea, dysentery, food poisoning, typhoid and paratyphoid fevers are at the top list of Diseases Under Surveillance (Notifiable Diseases). These are all food-borne diseases and WHO had shown that each year some 1500 million episodes of diarrhoea occur in children under the age of five and over three million die as a direct result. It has been estimated that up to 70 percent of diarrhoeal diseases may be caused by contaminated food. This also includes the water used in the preparation of food, washing of hands, food and utensils [7].

Table 3. Distribution of bacterial pathogens from different age groups of children attended the Yangon Children's Hospital during 2001

Age group (months)	Tested males	Pathogens isolated	Tested females	Pathogens isolated	Total
<6	6	A.hydrophila P. shigelloides S. sonnei V. cholerae O1 = 4 cases	3	nil	4 / 9 (44.44)
6-12	9	S. dysenteriae V. cholerae 0139 0114 K 90 0128 K 67 0159 K + = 5 cases	11	S. sonnei V. cholerae 0139 028a/cK73 0114K90 = 4 cases	9/20 (45)
13-24	13	A. hydrophila 027 K + 0119 K 69 0148 K + 0157K + 0159 K + = 6cases	11	V. dysenteriae 01 027K+ 0157K+ = 3 cases	9/24 (37.50)
25-36	5	S. sonnei V. cholerae 01 = 2 cases	2	S. dysenteriae A = 1 case	3/5 (60)
37-48	0		1	V. cholerae 01 = 1 case	1/1
48-60	1	V. cholerae 01 = 1 case	0		1/1
Total	34	18 (52.94)	28	9 (32.14)	27/62(43.55)

Figures in parenthesis denote percentages

Table 4. Association of bacterial pathogens isolated from stool samples and from vegetables in different townships

Sr. No.	Townships	Cases	Pathogens isolated from	
			Diarrhoeic children	Vegetables
1	Ahlone	5	*E. coli* O159K+*	*E. coli* O25K+,O86K62, O142K+, O148K+
2	Dagon (North)	2	*A. hydrophila* *V. cholerae* O1	Not tested
3	Insein	4	*S. dysenteriae* *V. cholerae* O1	*E. coli* O28 a/c, O111K58, O126K71 *S. dysenteriae*
4	Kamayut	5		*E. coli* O111 K58, O119K69, O146K89 *V. cholerae* 1
5	Kyauktan	3	*S. sonnei* (2 cases) *V. cholerae* O139	Not tested
6	Mingaladon	4	*E. coli* O157 K+ *S. sonnei*	*E. coli* O126K71, O142K+ *S. sonnei*
7	Papedan	4	*E. coli* O157K+; O159K+* *V. cholerae* O1	*E. coli* O28a/cK73, O119K69
8	Sanchaung	3	*E. coli* O128K67 *V. cholerae* O1	*E. coli* O28a/cK73 O111K58
9	Tamwe	7	*E. coli* O27K+; O28a/cK73;O148K+* *S. dysenteriae* A *V. cholerae* O139	*E. coli* O28 a/cK73 O111K58 O125K+ *V. cholerae* O1
10	Thaketa	13	*E. coli* O27K+; O119K69 *P. shigelloides* *V. cholerae* O1	*E. coli* O6K15 O26K15, O86K62,O146K69 *Salmonella* spp.
11	Thingangyun	6	*E. coli* O114K90 *(2 cases) *A. hydrophila*	
12	Others	6	nil	nil
	Total	62	27 cases	31 samples

Table 5. Total bacterial count in different vegetables after washing with 2 litres of clean water (initial count= uncountable organisms/ml or >1800MPN/100ml)

Myanmar	English	Scientific name	Weight (grams)	Washing times	Count or g/ml
1. Gazun	water convolvulus	*Ipomaea aquatica*	101.8	8	350
2. Kyethunmeik	onion tops	*Allium cepa*	49.1	7	375
3. Khayanthi	egg plant	*Solanum melongena*	80.2	6	400
4. Gobidouk	cabbage	*Brassica oleracea*	140.4	6	150
5. Hsalat	lettuce	*Lactuca sativa*	9.7	8	40
6. Nannanpin	coriander	*Coriandum sativum*	42	11	150
7. Pinzein	basil	*Ocimum sanctum*	31.3	6	250
8. Pusinan	mint	*Mentha arvensis*	15	8	280
9. Mounlaouhpyu	radish		78	11	515
10. Mounlapin	radish plant		32	11	UC
11. Myinkhwa	pennywort	*Hydrocotyl asiatica*	32.2	6	250
12. Shaukyet	lemon	*Citrus hystrix*	16.2	6	36
13. Thayetkin		unknown	2.2	9	400
14. Thakhwathi	Cucumber	*Cucumis sativus*	92.6	6	350

UC = Uncountable MPN = Most Probable Number

Access to good quality, safe and nutritious food is considered as basic right of the people. Consumption of unsafe, contaminated food leads to food-borne diseases which cause considerable morbidity and mortality. In many countries, the diseases transmitted by food are commonly referred to as food poisoning and are characterized by abrupt onset of gastro-intestinal disturbances *viz.* abdominal pain, vomiting and diarrhoea. The foods most commonly involved in food-borne disease are meat and meat products, poultry, eggs, milk and milk products, sweet meatsand rice preparations. In Myanmar, cholera is the most frequent food-borne outbreak. Every year there are pockets of endemic or epidemic outbreaks occurring throughout the country. The main cause is due to contamination of food during preparation or storage before serving. Incidence of infections caused by some pathogens *viz. Bacillus cereus, Staphylococcus aureus, Clostridium perfringens* is probably very high, however, as the disease are often self limiting, they receive little attention to public health services, unless there is mass involvement. Typhoid and paratyphoid food-borne outbreak is also common [7]. Our data, mainly concised on Enterobacteriaceae and Vibrionaceae, had shown that organisms from these two families were isolated from all the three sources. It revealed that potential source of infection among diarrhoea cases of children came from foods which were available easily in their environment. Thus, environmental sanitation and personal hygiene still play important roles to eradicate infections especially among young children.

The recommendations from these findings are:

1. Do not eat vegetables without washing.
2. As it may be difficult to make free of pathogens from vegetables, children should not be allowed to touch things before washing to be cooked.
3. Do not believe that all milk are well pasteurized even labelling.
4. Eat fresh things (just after cooking) as much as possible.
5. Do not eat leftover dishes especially after keeping for more than 6 hours.
6. Fresh yogurt is most suitable for consumption.
7. Always prepare for good sanitation.

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