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Bacterial isolates from non-processed fruit juices of Yangon

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A total of 70 samples of 7 different types of non-processed fruit juices (tamarind, water melon, sugar cane, pine apple, papaya, lime and black agar) and 15 different processed samples were collected randomly from six townships of Yangon from October 2002 to March 2004. All the samples of pine apple, water melon, papaya, sugar cane, and black agar drinks examined in the current study were found to be contaminated with coliforms (100%). In contrast, 40% and 10% of tamarind juice samples were contaminated with coliforms and faecal coliforms respectively. All samples of papaya, sugar cane and black agar juices were contaminated (100%) with faecal coliforms, while pine apple juice showed only 70% contamination. *Escherichia coli, Klebsiella aerogenes, Salmonella spp., Shigella boydii, Proteus mirabilis, Vibrio cholerae, Staphylococcus epidermidis* and *Staphylococcus aureus* were isolated and identified from the non-processed soft drinks. However, no bacteria was isolated from lime juice samples. The water that was used for preparation of the drinks was found to be contaminated with bacterial pathogens. The antibiotic susceptibility patterns of the isolated enteropathogenic *E. coli* (EPEC) showed that they were resistant to ampicillin and carbenicillin (63.64% each), and *Vibrio* species to ampicillin (80%). Plasmids were isolated from 16 out of 32 *E. coli* and 14 out of 15 *Vibrio* species. The size of plasmids was estimated ranging from 20 to 23.13 kilo base pairs.

INTRODUCTION

Ingestion of contaminated food or water is considered to be the principle mode of transmission of enteric pathogens [1]. Some bacterial species are always present and may constitute the normal flora of natural water. Opportunistic bacterial pathogens, naturally present in the environment are not usually regarded as pathogens but are able to cause diseases in people with impaired local or general immune mechanism. Safe water must be free from microorganisms and chemical substances. Fruits and vegetables can also be contaminated in areas where the lands are fertilized with human faeces [2]. Much attention had been paid to the quality, contamination and spoilage of soft drinks [3]. Contamination may occur through various sources from vending machines until they are sold to consumer. Bacteria can survive in ice of popular drinks [4]. Standard quality control protocols should be implemented for drinking water distribution networks [5] and relationship of drinking water quality and infectious diarrhoeal diseases was also reported [6,7]. The present study was carried out to demonstrate the occurrence of bacterial contamination of locally made, non-processed soft drinks in Yangon area, Myanmar. The current research was undertaken to isolate and identify bacteria from non-processed and processed local soft drinks of Yangon, Myanmar to help in food quality control and safety.

The specific objectives were:

- to detect the presence of coliforms and faecal coliforms in the processed and non-processed local soft drinks.

- to determine the distribution pattern of bacteria in the soft drinks tested township-wise. and
- to identify the serotypes and the antibiotic susceptibility of the isolated bacterial strains and to do the plasmid analysis of the bacterial isolates.

MATERIALS AND METHODS

Period of study was from October 2002 to March 2005.

Collection of samples

Seventy different non-processed drink samples (tamarind, water melon, sugar cane, pine apple, papaya, lime and black agar) and 15 different processed soft drinks (tamarind juices, lychee, milk juices, and soybean milk) were collected without ice from shops and vendors located at various townships (Hlaing, Insein, Kamayut, Lanmadaw, Tamwe and Sanchaung) of Yangon area.

Determination of coliforms and faecal coliforms in the soft drinks

The presence of coliforms and faecal coliforms was determined by multiple tube method and by membrane filtration technique as described in WHO , 1997 [2].

Isolation, identification and characterization of bacteria from the soft drinks

Based on the methods described in WHO [8], total bacterial counts and specific bacterial counts were determined. The bacterial isolates were identified based on the biochemical and serological tests [9].

Serological characterization of bacterial isolates

Slide agglutination tests were carried out using respective antisera of *E. coli, Salmonella, Shigella* and *Vibrio* spp., provided by Denka-Seika Company Limited, Japan, Difco, USA or DMR-001.

Antibiotic susceptibility testing

All EPEC isolates and *Vibrio* isolates obtained were subjected to antibiotic susceptibility testing by the method of Kirby-Bauer [10].

Plasmid DNA analysis of bacterial isolates

Rapid isolation of plasmid DNA was carried out by a modified Birnboim procedure [11] as described by Schleif and Wensink [12].

RESULTS

Processed soft drinks

Pathogenic bacteria was not isolated from all test samples.

Presumptive total coliform counts from the non-processed soft drinks samples

All the samples of water melon, papaya, black agar and pine apple juices were contaminated with coliforms. The coliform bacteria were absent only in lime juices. In contrast, they were all very high in papaya, water lemon, black agar and sugar cane juices (Table 1). The coliform counts in water melon, papaya, black agar, pine apple and sugar cane juices did not show any significant differences, but the counts in tamarind and lime juices when compared with previous samples showed significant differences ($p<0.04$).

Presumptive faecal coliform (FC) count from the non-processed soft drinks samples

All water melon, papaya, black agar and sugar cane samples (100 percent) were contaminated with faecal coliforms. Faecal coliforms were identified from 3 samples of pine apple. In contrast, only one sample of tamarind juice was contaminated with faecal coliforms. Faecal coliforms were absent in all the 10 samples of lime juice. The faecal coliforms counts from all different samples of juices ranged from 6 to >1800 MPN/100ml (Table1). Similar findings were obtained as in coliform count and the counts in pine apple, tamarind and lime were found to be significantly different (p<0.04) when compared to the counts from water melon. The water samples used for the preparation of the juices were found to be contaminated with coliforms (85.7%) and also with faecal coliforms (33.3%).

Distribution of bacterial isolates in the non-processed soft drinks

Presence of *E. coli* was detected in 71.4% of non-processed soft drink samples and 85.7% of water samples used for preparation of the juices from various shops. *Klebsiella aerogenes* was found in 12 samples (17.1%), *Staphylococcus aureus* in 8 samples (11.4%), *Staphylococcus epidermidis* in 6 samples (8.57%). *Salmonella* species in one sample (1.4%), and V*ibrio cholerae* in 5 samples (7.1%) of the non-processed drinks. *Shigella boydii* was isolated from 3 samples (4.3%) of juices and only one sample (4.8%) of water which was used to make the soft drinks was contaminated with *Shigella* species (Table 2).

Table 1. Presumptive total coliform and faecal coliform counts in non-processed soft drinks samples

Sr. No.	Fruit juices soft drinks	Number of samples contaminated with coliforms and faecal coliforms		Range of coliform and faecal coliform counts (MPN/100ml)	
		Coliforms	Faecal coliforms	Coliforms	Faecal coliforms
1	Tamarind (n=10)	4	1	6-84	6 -9
2	Water melon (n=10)	10	10	>1800	>1800
3	Papaya (n=10)	10	10	>1800	6- >1800
4	Black agar (n=10)	10	10	>1800	12- >1800
5	Pine apple (n=10)	4	3	12- >1800	6- >1800
6	Sugarcane (n=10)	10	10	>1800	6- >1800
7	Lime (n=10)	0	0	<2	<2
	Total (n=70)	48 68.56 %	44 8.56 %	<2- >1800	<2 - >1800
8	Water (n=21)	18 (85.71%)	7 (33.33%)	12- >1800	6- >1800

Table 2. Bacteria isolates from various kinds of non-processed soft drinks

Name of non-processed soft drinks	*Escherichia coli*	*Klebsiella aerogenes*	*Salmonella* species	*Shigella boydii*	*Proteus mirabilis*	*Vibrio cholerae*	*Staphylococcus epidermidis*	*Staphylococcus aureus*	Total bacterial isolates
Tamarind (n=10)	4	2	0	1	0	2	1	0	8
Water melon (n=10)	9	0	0	0	2	2	2	2	17
Papaya (n=10)	10	1	0	1	1	0	2	2	17
Black agar (n=10)	10	2	1	0	0	1	0	1	15
Sugar cane (n=10)	9	5	0	1	1	0	1	3	20
Pine apple (n=10)	8	2	0	0	1	0	0	0	11
Lime (n=10)	0	0	0	0	0	0	0	0	0
Total (n=70)	50 (71.43)	12 (17.14)	1 (1.43)	3 (4.28)	5 (7.14)	5 (7.14)	6 (8.57)	8 (11.43)	88
Water (n=21)	18 (85 . 71)	1 (4. 76)	0	1 (4. 76)	2 (2. 86)	0	0	0	22

Figures in parenthesis denote percentages of the bacterial isolates in non-processed soft drinks

The bacterial isolates that were identified from the non-processed soft drinks obtained from different localities of the Yangon city are detailed in Table 3. Serology showed that of 125 isolates of *E. coli*, 12 different types of *E. coli* were serogrouped; out of these, 7 isolates were O55K59, 5 isolates were O128K67, and 3 isolates were O26K60 .The other serogroups were: O6K15, O28K73, O78K80, O114K90, O126K71, O127K63, and O144Kx2 (Table 3).

Table 3. Places of collection and species of bacterial detected in non- processed fruit juices and water used for preparation

Place	Tamarind n=10	Water melon n=10	Sugarcane n=10	Pineapple n=10	Papaya n=10	Black agar n=10	Water n=21
Hledan	*E. coli* (2) O6K15, O28K73 *Klebsiella* spp. *S.epidermidis*	*E. coli* (2)	*E. coli* (4) O26K60, O55K59 O128K67, *Klebsiella*(3) *S. aureus*	*E. coli* (3) *Klebsiella*(2)	*E. coli*	*E. coli* (2) *Klebsiella* spp.	*E. coli* (5) O26K60
Insein	*E. coli*	*E. coli* *Proteus* spp.	*E. coli* (1), O55K59 O128K67, *Proteus* spp. *Vibrio* spp.	*E. coli* (2)	*E. coli* *Klebsiella* spp. *S. aureus* *S.epidermidis*	*E. coli* (2) 055K59, 0114K90 0144Kx2 *Klebsiella* spp. *Salmonells* spp *Vibrio* spp.	*E. coli*
Tamway	*Shigella* spp. *Vibrio* spp.	NT	NT	*E. coli*	*E. coli* *Shigella* spp. *S.epidermidis*	*E. coli* *S. aureus*	*E. coli* (2) *Shigella* spp.
Sinmaleik	*Klebsiella* spp	NT	NT	*E. coli* *Proteus* spp.	NT	NT	*E. coli* (2) *Proteus* spp.
Hlaing-yadanar	NT	*E. coli* (4), O55K59 O126K71, O128K67 *S.epidermidis,* *Vibrio* spp.	NT	NT	*E. coli* (2) 055K59(2) *Vibrio(1)*	NT	*E. coli*
Kyundaw	*Vibrio* spp.	NT	*E. coli,* *Vibrio* spp. *S.epidermidis*	NT	not isolated	*E. coli*(2) 026K60 055K59 *Vibrio* spp.	*E. coli* (2)
10th mile	*E. coli*	*E. coli,* O128K67 *S. aureus,* *S.epidermids* *Vibrio* spp.	*E. coli,* *Vibrio* spp. *Klebsiella*(1), *Shigella* spp.	not isolated	*E. coli* *Vibrio* spp.(2)	*E. coli*	*E. coli* (2) O78K80
Myenigone	NT	*Proteus* spp. *S. aureus,* *Vibrio* spp.	*E. coli*(2) *Klebsiella* *S. aureus* (2)	not isolated	*E. coli* (3). *Proteus*	*E. coli* 0128K67	*E. coli* (2)., *Klebsiella* spp. *Proteus* spp
Lanmadaw	NT	*E. coli* O127K63	NT	*E. coli*	*E. coli,* *S. aureus*	*E. coli*(2) 0128K67 *Vibrio* spp	*E. coli*
Total	10	18	23	11	20	17	22

NT=Not tested

Antibiotic sensitivity/resistance of the bacterial isolates

Data show that most of the isolates of *E. coli* were resistant to ampicillin, carbenicillin and amikacin while they were very sensitive to chloramphenicol, tri-methoprim and tetracycline. Most of the isolates of *Vibrio* spp. exhibited resistance to ampicillin, tetracycline and carbenicillin while they showed sensitivity to chloram-phenicol, carbenicillin, trimethoprim and amikacin (Table 4).

Plasmid profile

Plasmid analysis of 32 isolates of *E. coli* was done and plasmids were detected in 16 of the isolates while absent in others. All the plasmids were large in size (20-23kbp). Different strains of *E. coli* exhibited different sized plasmids probably reflecting the differences in the strains and also in fruit sources. The *E. coli* serotypes seemed to possess the largest sized plasmids. Altogether 16 isolates of *Vibrio* species were screened for the presence of plasmids and 14 isolates carried plasmids. All plasmids were estimated to be similar in size (equal to or greater than 23.13 kbp) Isolates 6 and 9 have been isolated from different fruits and localities and they exhibited the presence of large sized plasmids.

Table 4. Antibiotic susceptibility of *E.coli* (n=33) and *Vibrio* species (n=15) isolated from non-processed soft drinks samples

Susceptibility to antibiotics	Antibiotics					
	Ampicillin	Amikacin	Chloramphenicol	Carbenicillin	Trimethoprim/ sulfamethoxazole	Tetracycline
E. coli Resistant	21 (63.64)	14 (42.42)	4	21 (63.64)	8 (24.24)	3
Intermediate resistant	2	16	0	0	0	15
Moderately susceptible	8	0	0	4	22	0
Susceptible	2	3	29	8	14	15
Total *E. coli*	33	33	33	33	33	33
Vibrio species Resistant	12 (89.00)	2	2	3 (20.00)	3 (20.00)	5 (33.33)
Intermediate resistant	0	8	0	5	0	8
Moderately susceptible	1	0	0	1	3	0
Susceptible	2	5	13	6	9	2
Total *Vibrio* species	15	15	15	15	15	15

Figures in parenthesis denote percentages

DISCUSSION

The present study has shown that none of the processed soft drinks were contaminated with bacteria indicating that all the 15 different processed soft drinks were processed properly.

In contrast, most of the non-processed soft drink samples tested in the current study had shown that 50-100% were contaminated with coliforms and 10-100% with faecal coliforms. *Escherichia coli* was isolated from 40% of the samples of tamarind juices and from 80-100% of pine apple, water melon, sugar cane and papaya juices. Moreover, *E. coli* was isolated from 86% of water which was used to prepare the soft drinks. The reasons for these might have been due to the ubiquitous presence of *E. coli* in the localities and the hands and intestine of man; for example, about 200 to 400 million *E.* coli are reported to be excreted per day per person [13].

Other sources of contamination could be the handlers who prepare the drinks. They might have contaminated the drinks with *E. coli* or other coliforms or infectious bacteria. These might be one of the reasons for the products to be contaminated with *E. coli*. Surprisingly, *E. coli* could not be isolated from the freshly prepared lime juices probably due to the constituents present were antibacterial. Another reason for the low isolation rate of *E. coli* from tamarind juices and lime juices might be due to the low acidity of these drinks. The pH of tamarind juices ranges from 4-5 whereas the lime juices have a pH of 2. In contrast, the pH of other juices and soft drinks were in the range of 6-8. Most bacteria cannot thrive at the low pH level as most bacteria prefer a slightly alkaline pH of between 7.2 and 7.6 for their survival and proliferation [1, 13].

Plasmid analysis provides a useful tool, primarily to classify organisms at the subspecies level for epidemiologic investigations [14]. Plasmids of *E. coli* and *Vibrio cholerae* from this study showed similarities with those of the strains isolated from clinical sources. Thus, the spread of infectious bacterial in these juices might be due to the improper sanitation in that environment.

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