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Distribution of coliforms , faecal coliforms and enteropathogenic *E. coli* (EPEC) in fried rice and water samples from street vendors of Yangon and detection of bacterial toxins

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The contamination of coliforms, faecal coliforms and enteropathogenic *Escherichia coli* (EPEC) was tested in 45 fried rice samples collected from different street vendors of three townships from Yangon area. Toxin produced by *E. coli* was also studied. High total bacterial count of 10^4 to $>10^6$ was recorded from 42 samples of fried rice. Among them, coliforms, faecal coliforms and EPEC were isolated from 32 (71.11%) samples, 15 (33.33) samples and 29 (64.44 %) samples respectively. The serogroup O55K59 was the commonest pathogen isolated from 11 samples (24.44 %). The other serogroups encountered were O1K51 (from 4 samples), O26K60 (from 3 samples), O25K+ and O125K70 (from 2 samples each). Other serogroups isolated were O27K+, O44K74, O86K62, O111K58, O114K90, O128K70 and O136K78 (from one sample each). Enterotoxigenic *E. coli*–heat-labile (ETEC-LT) toxin and verotoxin (VTEC) were detected from 2 samples (4.44%) and from one sample respectively. Similarly, coliforms, faecal coliforms and EPEC were isolated respectively from 39 (86.66%), 21 (46.67%) and 23 (51.11 %) water samples used for multipurpose in those shops. The serogroup O55K59 was commonly found with the isolation rate of 11 (24.44%); O119K69 was isolated from 2 samples and the other serogroups such as O1K51, O26K60, O44K74, O86K62, and O128K67 were also detected . ETEC-LT toxin was detected from one sample (2.22%) of water only.

INTRODUCTION

Many microorganisms previously unrecognized as food-borne or harmful are emerging as human pathogens transmitted by food. Within the past decade, the epidemiology of microbial food-borne diseases has changed, not only because of human population's increasingly susceptibility to diseases and of changing life styles (including more adventurous eating, more convenience foods, and less time devoted to food preparation) but also because of the emergence of newly recognized food-borne pathogens [1, 2]. In developing countries, a great number of ready-to-eat food is sold on the streets. The term "street food" refers to a wide variety of ready-to-eat foods and beverages sold, and sometimes prepared, in public places. As with fast food, the final preparation occurs when meals are ordered by customers. Street food may be consumed where it is purchased or can be taken away and eaten elsewhere. The consumption of street food is common in many countries where unemployment is high, salaries are low, work opportunities and social programs are limited, and where urbanization is taking place. Street food vendors benefit from a positive cash flow, often evade taxation, and can determine their own working hours. By selling snacks, complete meals and refreshments at relatively low prices, they provide an essential service to workers, shoppers, travelers, and people with low incomes. People who depend on such food are often more interested in its convenience than in questions of its safety, quality and hygiene. The hygienic aspects of vending operations are a major source of concern for food control officers. For example, stands are often crude structures, and running water may not be readily available. Also toilets and adequate washing facilities are rarely available. The washing of hands, utensils, and dishes is often done in buckets or bowls. Disinfection is not usually carried out, and insects and rodents may be attracted to sites where there is no organized sewage disposal [3,4]. Finally food is not adequately protected from flies and refrigeration is usually unavailable. The presence of coliforms and faecal coliforms in food and water is an indicator for contamination. Water and food always serve as vehicles in transmission of contaminated bacteria. Thus, this study was carried out to investigate the contaminated bacteria in food and water from street vendors of Yangon area.

Objective

To investigate the presence of coliforms, faecal coliforms and enteropathogenic *Escherichia coli* (EPEC) in fried rice and water sold by street vendors of Yangon area.

Specific objectives

1. To determine the presence of coliforms and faecal coliforms in fried rice and water
2. To isolate *Escherichia coli* from fried rice and water
3. To identify enteropathogenic *Escherichia coli* (EPEC)
4. To define the enterotoxin produced by *E. coli* isolates.

MATERIALS AND METHODS

Tested samples

Forty five samples of fried rice (approx. 500 grams) sold in street vendors and water samples (one litre) used for multipurpose from respective shops were collected aseptically from three townships of Latha, Yankin and Dagon new satellite town from 25-1-05 to 16-5-05.

Sample preparation

Twenty grams of fried rice were soaked in phosphate buffer (PBS) and homogenized with the aid of stainless steel homogenizer (Ace homogenizer, Nihonseiki kaisha Ltd.). Serial 10-fold dilutions were carried out in 90 ml PBS to obtain up to 10^5 dilutions aseptically [5].

Total bacterial count

It was carried out by the method of Miles and Misra, 1930 [6].

Determination of coliforms and faecal coliforms

It was carried out by the multiple tube method as well as by Millipore membrane filtration method [7]. Isolation, identification and characterization of bacteria from fried rice were carried out by the methods described in WHO, 1982 [8] and by Lennette, Balows, Hausler and Shadomy, 1985 [9] and serological tests by Ewing, 1986 [10].

Enterotoxin assays

They were carried out for *Escherichia coli* heat labile enterotoxin (LT) and verotoxin detection using VET-RPLA kit (Oxoid TD-920) and VET-RPLA kit (Oxoid, TD-0960A) which are reverse passive latex agglutination test. The isolates were cultured in Casamino Acid Yeast Extract (CA-YE) broth at 37°C overnight in a shaking waterbath. The culture supernatant was used by mixing with polymer latex particles sensitized with purified rabbit antiserum which is reactive with *E. coli* heat labile toxin or with polymer latex particles sensitized with purified rabbit antiserum which is reactive with *E. coli* VT1 or VT2.

RESULTS

Determination of total bacterial count on nutrient agar

The total bacterial count of fried rice varied from different samples and within three townships. Most of the samples i.e. 27 samples (60.00 %) showed that their count fell into between 2.0 x 10^4 cfu /gm to 10^5 cfu/gm. Only three samples of fried rice showed <10^4 cfu/gm. Then 5 and 10 samples of fried rice had high bacterial count which fell into the range of 10^5 to 10^6 and >10^6 cfu/gm respectively. The minimum count was<10^4 and the maximum count was >10^6cfu / gm (Table1).

Table 1. Total Bacterial Count in fried rice from three townships

Sr No.	Count CFU/gm	Latha	Yankin	Dagon	All townships
1	< 10^4	1 (6.67)	1 (6.67)	1 (6.67)	3 (6.67)
2	10^4 to 10^5	10 (66.67)	7 (46.66)	10 (66.67)	27 (60.00)
3	10^5 to 10^6	3 (20.00)	1 (6.67)	1 (6.67)	5 (11.11)
4	>10^6	1 (6.67)	6 (40.00)	3 (20.00)	10 (22.22)
Total samples		15	15	15	45

Figures in parenthesis denote percentages

Determination of coliforms and faecal coliforms by Multiple Tube method

Coliforms were identified from 66.67%, 66.67% and 80% of fried rice from Latha, Yankin and Dagon respectively. Similarly, faecal coliforms were isolated from 26.66%, 53.33% and 20% of the samples from their respective townships. In the same study 10 (66.67%), 14 (93.33%) and 15 (100%) number of the water samples from Latha, Yankin and Dagon were contaminated with coliforms respectively. Also, 7 (46.67%), 9 (60.00%) and 5 (33.33%) samples of water were contaminated with faecal coliforms from those townships respectively (Table 2).

Table 2. Distribution of coliforms, faecal coliforms and enteropathogenic *Escherichia coli* (EPEC) from fried rice and water samples

Townships	Fried rice	Water
LATHA n= 15 each	Coliforms 10(66.67%) Faecal Coliforms 4 (26.66%) EPEC 9 (60.00%) ETEC-LT toxin 1 (6.67%) VTEC = Nil	Coliforms 10(66.67%) Faecal Coliforms 7(46.67%) EPEC 8 (53.33%) ETEC-LT toxin 1 (6.67%) VTEC = Nil
YANKIN n= 15 ecah	Coliforms 10 (66.67%) Faecal Coliforms 8 (53.33%) EPEC 10 (66.67%) ETEC-LT toxin 1 (6.67%) VTEC = Nil	Coliforms 14 (93.33%) Faecal Coliforms 9 (60.00%) EPEC 8 (53.33%) ETEC-LT toxin Nil VTEC = Nil
DAGON n= 15 each	Coliforms 12 (80.00%) Faecal coliforms 3 (20.00%) EPEC 10 (66.67%) ETEC-LT toxin Nil VTEC-VT_2 1 (6.67%)	Coliforms 15 (100%) Faecal coliforms 5 (33.33%) EPEC 7 (46.66%) ETEC-LT toxin Nil VTEC = Nil
ALL TOWNSHIPS n=45 each	Coliforms 32 (71.11%) Faecal coliforms 15 (33.33%) EPEC 29 (64.44%) ETEC-LT toxin 2 (4.44%) VTEC 1 (2.22%)	Coliforms 39 (86.66%) Faecal coliforms 21 (46.67%) EPEC 23 (51.11%) ETEC-LT toxin 1(2.22%) VTEC = Nil

Isolation of Enteropathogenic Escherichia coli (EPEC) ,ETEC & VTEC from fried rice and water samples from three townships and detection of enterotoxin

Enteropathogenic *Escherichia coli* (EPEC) was isolated from 29 (64.44%) samples of fried rice and 23 (51.11%) samples of water. These were 9 (60%) from Latha:, 10 (66.67%) from Yankin and 10 (66.67%) from Dagon. Similarly 8 (53.33%) number of water samples each from Latha and Yankin were contaminated with EPEC. ETEC-LT toxin from 2 samples (4.44%)

Table 3. Isolation of different serogroups from three townships

Sero-groups	Latha		Yankin		Dagon		Total	
	Food	Water	Food	Water	Food	Water	Food	Water
O1 K51	1	1	2	0	1	3	4	4
O25 K+	1	0	1	0	0	0	2	0
O26 K60	0	0	1	1	2	0	3	1
O27 K+	0	0	0	0	1	0	1	0
O44 K74	0	0	0	0	1	1	1	1
O55K59	6	6	3	3	2	2	11	11
O86 K62	0	0	1	1	0	0	1	1
O111 K58	0	0	0	0	1	0	1	0
O114 K90	0	0	0	0	1	0	1	0
O119 K69	0	0	0	1	0	1	0	2
O125 K70	0	0	2	2	0	0	2	2
O128 K67	0	1	0	0	1	0	1	1
O136 K78	1	0	0	0	0	0	1	0
Total	9 (60.00)	8 (53.33)	10 (66.67)	8 (53.33)	10 (66.67)	7 (46.66)	29 (64.44)	23 (51.11)

Figures in parenthesis denote percentages

and VTEC (VT2) from one sample of fried rice (2.22%) were also detected (Table 2). The serogroup O55K59 was the commonest pathogen isolated from 11 samples (24.44%). The other serogroups encountered were O1K51 (from 4 samples), O26K60 (from 3 samples), O25K + and O125K70 (from 2 samples each). Other serogroups isolated were O27K+, O44K74, O86K62, O111K58, O114K90, O128K67 and O136K78 (from one sample each) (Table 3). Similarly, 39 (86.66%), 21 (46.67%) and 23 (51.11 %) number of coliforms, faecal coliforms and EPEC respectively were isolated from water samples used for multipurpose in those shops. Similar serogroups such as O55K59 was found to be common with the isolation rate of 11 (24.44%). O119K69 was isolated from 2 samples and the other serogroups such as O1K51, O26K60, O44K74, O86K62, and O128K67 were also detected (Table 3). ETEC–LT toxin was detected from one sample (2.22%) of water.

Distribution of coliforms and faecal coliforms in fried rice and water samples

This study showed that coliforms were solated from 71.11 percent and 86.67 percent of fried rice and water samples respectively. Faecal coliforms were identified from 33.33 percent and 46.67 percent of fried rice and water samples respectively (Table 2).

DISCUSSION

Microbial pathogenicity has been defined as the structural and biochemical mechanisms whereby microorganisms cause disease. Infection may imply colonization, multiplication, invasion or adhesion of a pathogen on or within a host, but disease (infectious disease) is used to describe an infection that causes significant overt damage of the host. *Escherichia coli* isolates that bear mannose-resistant hemagglutinins and related adhesions, are associated with two common diseases: urinary tract infection and diarrhea. Epidemiological studies suggest that children and pregnant women are prone to recurrent or persistant infections caused by these organisms. Diffuse-adhering *Escherichia coli* (DAEC) is a putative cause of diarrhea in Mayan children in Mexico [11]. It was noted that *E. coli* was isolated from 50%, 50% and 30 % of children with cystitis, protracted diarrhea and pyelonephritis in pregnant women respectively. The relationship between the presence of bacterial virulence and the severity of urinary tract infection (UTI) was shown by Blanco *et al.*, 1996 [12]. It was reported that bottled water may cause 10% of food poisoning cases [13]. A close relationship between presence of CFAs and determinate serotypes was reported [14]. In our study, *E. coli* and EPEC were isolated from both food and water in three areas. The study revealed the importance of the contaminated bacteria either in food or water with high risk to infect children and adults. The pathogenic bacteria can cause gastroenteritis and urinary tract infection. Thus, safety of food and water is always essential.

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