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Contamination of faecal coliforms in ice cubes sampled from food outlets in Kubang Kerian, Kelantan

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Abstract. The use of ice cubes in beverages is common among patrons of food outlets in Malaysia although its safety for human consumption remains unclear. Hence, this study was designed to determine the presence of faecal coliforms and several useful water physicochemical parameters viz. free residual chlorine concentration, turbidity and pH in ice cubes from 30 randomly selected food outlets in Kubang Kerian, Kelantan. Faecal coliforms were found in ice cubes in 16 (53%) food outlets ranging between 1 CFU/100mL to >50 CFU/100mL, while in the remaining 14 (47%) food outlets, in samples of tap water as well as in commercially bottled drinking water, faecal coliforms were not detected. The highest faecal coliform counts of >50 CFU/100mL were observed in 3 (10%) food outlets followed by 11-50 CFU/100mL and 1-10 CFU/100mL in 7 (23%) and 6 (20%) food outlets, respectively. All samples recorded low free residual chlorine concentration (<0.10mg/L) with the pH ranging between 5.5 and 7.3 and turbidity between 0.14-1.76 NTU. Since contamination by faecal coliforms was not detected in 47% of the samples, tap water and commercially bottled drinking water, it was concluded that (1) contamination by faecal coliforms may occur due to improper handling of ice cubes at the food outlets or (2) they may not be the water sources used for making ice cubes. Since low free residual chlorine concentrations were observed (<0.10mg/L) in all samples as well as in both tap water and commercially bottled drinking water, with the pH ranged between 5.5-7.3, ineffective disinfection of water source as a contributing factor to such high counts of faecal coliforms in ice cubes also could not be ruled out. Therefore, a periodical, yet comprehensive check on the food outlets, including that of ice cube is crucial in ensuring better food and water for human consumption.

INTRODUCTION

Consumption of iced-beverages is popular in tropical countries like Malaysia due to hot weather condition especially during the dry season. Since ice cubes added to beverages melt and thus directly ingested while drinking, they should be of the same quality as drinking water. However, many studies conducted in other countries reported that manufactured edible ice cubes use in foods and drinks too could be a cause for human health concern (Nichols *et al.*, 2000; Falcao *et al.*, 2004; Lateef *et al.*, 2006). Such studies revealed that *Escherichia coli*, coliforms and a variety of microorganisms were present in ice cubes attributable to poor quality of source water used, lack of hygiene in the production process as well as improper handling. Furthermore, many cases of food poisoning have been associated with ice cubes (Wilson *et al.*, 1997; Falcao *et al.*, 2004), apart from the fact that ice-related nosocomial transmission of pathogens has also been reported (Graman *et al.*, 1997).

In Kelantan (Kubang Kerian), an eastern state of peninsular Malaysia, most of the ice cubes used by food outlets are supplied by small-scale ice cube vendors and the source of water used for making these ice cubes is generally unknown. It is pertinent to mention here that 26% and 10% of treated water samples in Kelantan contain unacceptable levels of total coliforms and faecal coliforms, respectively and periodic analysis reveals that it also contains high levels of iron, manganese and aluminium (Auditor General Report, 2009). Zaliha & Abdullah (2004) indicated that about 22% of the food premises in Kota Bharu, Kelantan had unsatisfactory score when assessed against the hygienic standard of food premises and micro-biological quality guideline provided by the District Health Office. Moreover, data obtained from the record unit of Hospital Universiti Sains Malaysia (HUSM), located in Kubang Kerian, Kelantan, revealed that during the year 2004 to 2008, 2457 patients had been admitted due to food and water borne diseases (unpublished data). Ironically, while having elaborate guideline to ensure the hygiene and safety of water and foods (Zaliha & Abdullah, 2004), analysis on ice cube has not been part of the routine checks in Kelantan. Lateef *et al.* (2006) further indicated that many countries do not have specific national microbiological guideline for ice. Hence, information on the safety of ice cube for human consumption remains lacking.

Therefore, this preliminary study aimed specifically at determining the presence of faecal coliforms and the several useful water physicochemical parameters viz. free residual chlorine concentration, pH and turbidity in ice cubes sampled from several food outlets in various locations in Kubang Kerian, Kelantan. The data obtained would be of applied value in enabling the local health authorities in improving the existing hygienic standard of food premises and microbiological quality of food, as well as designing the appropriate community awareness programmes.

MATERIALS AND METHODS

Sampling and exclusion criteria

This study was conducted in Kubang Kerian (a bustling new economic hub), located about 7 Km from Kota Bharu, Kelantan, Malaysia during July to September 2009. All the permanent food outlets numbering 100 located within the 2 Km radius of the town of Kubang Kerian, Kelantan (6º05N, 102º17E) was mapped and numbered (1-100) and out of these 100 food outlets, a total of 30 food outlets were randomly chosen. In this study, temporary food premises such as night markets and food stalls as well as permanent food outlets that did not sell iced-beverages were excluded. Samples of ice cubes were analyzed for the presence of faecal coliforms as well as the physicochemical parameters viz. free residual chlorine concentration, pH and turbidity. For comparison, specimens of tap water obtained within HUSM as well as the commercially bottled drinking water were also included in the analysis and they were referred as Control 1 and Control 2, respectively. Specimens of ice cubes (approximately 0.5 liter) were aseptically collected (Cheesebrough, 2006), placed in a sterile insulated cold box whose temperature was kept at about 5ºC, transported immediately to the laboratory and were analyzed within 2 hours of collection. Analysis was done in triplicates and the mean value was calculated.

Faecal coliform count

In the laboratory, the ice cube sample was allowed to melt under aseptic condition and once melted a 100 mL of sample was mixed thoroughly by inverting the bottle several times and then filtered through a sterile membrane filter (cellulose acetate, 0.45 µm pore size) with a diameter of 47 mm attached to a vacuum pump. Once the filtration was completed, the cellulose acetate membrane was placed centrally on a Mac Conkey agar and incubated at 44.5ºC for 24 hours. Upon completion, the plate was observed for the presence of small and smooth convex pinkish lactose fermenting colonies with 1-3 mm in diameter (Cheesebrough, 2006) and the number of these colonies was counted. When the colonies were found to be too numerous, such confluent growth was reported as 'too numerous to count' i.e. >50 CFU/100mL (Cheesebrough, 2006). The Eosin-Methylene Blue (EMB) agar and IMViC test were also utilized to verify the presence of faecal coliforms over other types of possible enteric pathogens.

Physicochemical analysis

Sample of ice cubes was analyzed for several physicochemical parameters viz. free residual chlorine concentration, pH and turbidity. Chemical analysis of the free residual chlorine concentration was carried out using the N, N-diethyl-p-phenylene diamine (DPD) method. In general, one DPD Free Chlorine Powder Pillow was added to a 10 mL sample of water, swirled vigorously and the colour of the solution would turn pinkish if free residual chlorine was present. The sample was immediately placed in a portable colorimeter (HACH DR/890) (HACH, Colorado, USA) and the result was recorded. In addition, the pH and turbidity (in Nephelometric Turbidity Units, NTU) of each sample was measured using a calibrated pH meter (model pH 211) (HANNA Instrument, Michigan, USA) and a turbidimeter (model 2100AN) (HACH, Colorado, USA), respectively.

RESULTS

The overall results on the hygienic standard of food premises and microbiological quality of food scoring grade among individual outlets, source of ice cubes, faecal coliform counts as well as the physicochemical parameters analysed in all ice cube samples are abstracted in Table 1. About 100 permanent food outlets had been identified within the 2 Km radius of Kubang Kerian town and 30 food outlets were randomly selected. When asked about the supply of ice cubes used in their food outlets, 29 out of 30 food operators claimed that they purchased them from commercial vendors; ice cubes were made in-house in one food outlet (No. 5) (Table 1). Although all permanent food outlets/premises are required by law to display their scoring grades on hygienic standard of food premises and microbiological quality of food, only one food outlet designated as 'No. 5' complied (Table 1). Our analysis revealed that ice cubes sampled from 16 (53%) out of 30 food outlets included in this study were contaminated with faecal coliforms measuring between 1 CFU/100mL to more than 50 CFU/100mL. On the other hand, contamination by faecal coliforms was not detected in the remaining 14 (47%) food outlets, in samples of tap water as well as in the commercially bottled drinking water. Alarmingly, samples of ice cubes in three (10%) food outlets were found to be grossly polluted with faecal coliforms (>50 CFU/100mL). In seven (23%) food outlets, samples of ice cubes contained between 11-50 CFU/100mL of faecal coliforms, while in six (20%) food outlets the faecal coliform counts ranged between 1-10 CFU/100mL. Interestingly, despite being classified as 'grade A' in its hygienic standard and microbiological quality of food score by the District Health Office as well as claimed to produce its own ice cubes for business, faecal coliform count of 4 CFU/100mL was detected in ice cubes in food outlet labeled as 'No. 5' (Table 1).

It was found that the free residual chlorine concentrations, pH and turbidity in all ice cube samples ranged between 0.01-0.07 mg/L, 5.5-7.3 and 0.14-1.76 NTU, respectively (Table 1). In tap water, the free residual chlorine concentration, pH and turbidity were consistently found at 0.04 mg/L, 6.5 and 3.02 NTU, respectively, while in the commercially bottled drinking water the same parameters were of 0.04 mg/L, 7.0 and 0.13 NTU, respectively (Table 1).

DISCUSSION

It has been indicated that total coliform count may not be useful in indicating the sanitary quality of water supplies, particularly in tropical countries since many bacteria of no sanitary significance occur in almost all untreated water supplies (World Health Organization, WHO, 2008). In contrast, faecal coliform count (sometimes called thermotolerant coliform organisms or *E. coli*) has been regarded as the most important indicator for faecal contamination in water (in this case, ice cubes) (Cheesebrough, 2006). As prescribed in the Guidelines for Drinking Water Quality 3rd Edition (WHO, 2008) as well as the Standard for Water and Packaged Drinking Water (Food Act 1983 (Act 281) & Regulations, 2004), *E. coli* or thermotolerant coliform bacteria must not be

Table 1. Cleanliness scoring grade, faecal coliform counts and physicochemical parameters observed in all ice cube samples

Food outlets	Hygienic standard of food premises and microbiological quality of food scoring grade given by the District Health Office	Source of ice cubes	Faecal coliform counts (CFU/100mL)	Free residual chlorine concentration (mg/L)	Turbidity (NTU)	pH
No. 1	Not displayed	Commercial vendor	Not detected	0.05	0.36	6.2
No. 2	Not displayed	Commercial vendor	Not detected	0.05	0.60	6.7
No. 3	Not displayed	Commercial vendor	3	0.03	0.26	6.2
No. 4	Not displayed	Commercial vendor	9	0.02	0.39	6.5
No. 5	Grade A (very clean)	In-house production	4	0.01	0.25	5.5
No. 6	Not displayed	Commercial vendor	Not detected	0.03	0.19	6.4
No. 7	Not displayed	Commercial vendor	Not detected	0.03	1.08	6.1
No. 8	Not displayed	Commercial vendor	Not detected	0.04	1.76	5.9
No. 9	Not displayed	Commercial vendor	Not detected	0.03	0.14	6.1
No. 10	Not displayed	Commercial vendor	2	0.02	0.64	6.2
No. 11	Not displayed	Commercial vendor	Not detected	0.05	0.20	6.0
No. 12	Not displayed	Commercial vendor	Not detected	0.05	0.31	5.9
No. 13	Not displayed	Commercial vendor	12	0.05	0.24	6.3
No. 14	Not displayed	Commercial vendor	>50	0.03	0.42	5.9
No. 15	Not displayed	Commercial vendor	Not detected	0.02	0.63	7.3
No. 16	Not displayed	Commercial vendor	28	0.04	0.60	6.2
No. 17	Not displayed	Commercial vendor	>50	0.07	0.61	7.0
No. 18	Not displayed	Commercial vendor	>50	0.06	0.41	5.5
No. 19	Not displayed	Commercial vendor	2	0.02	0.33	6.2
No. 20	Not displayed	Commercial vendor	14	0.04	0.52	6.1
No. 21	Not displayed	Commercial vendor	22	0.04	0.42	6.5
No. 22	Not displayed	Commercial vendor	Not detected	0.05	0.15	6.2
No. 23	Not displayed	Commercial vendor	20	0.05	0.33	6.3
No. 24	Not displayed	Commercial vendor	Not detected	0.04	0.38	6.1
No. 25	Not displayed	Commercial vendor	10	0.02	0.21	6.0
No. 26	Not displayed	Commercial vendor	4	0.03	0.21	6.5
No. 27	Not displayed	Commercial vendor	21	0.04	0.38	5.8
No. 28	Not displayed	Commercial vendor	Not detected	0.03	0.33	6.8
No. 29	Not displayed	Commercial vendor	Not detected	0.02	0.46	5.9
No. 30	Not displayed	Commercial vendor	Not detected	0.02	0.54	6.1

Grading for the hygienic standard of food premises and microbiological quality of food score: A (Very clean); B (Clean); C (Not clean)
For comparison: In tap water (HUSM) (Control 1): faecal coliforms were not detected; free residual chlorine concentration=0.04 mg/L; pH=6.5; turbidity=3.02 NTU. In commercially bottled drinking water (control 2): faecal coliforms were not detected; free residual chlorine concentration=0.04 mg/L; pH=7.0; turbidity=0.13 NTU

detectable in any 100 mL sample of water directly intended for drinking. Due to this reason, this present study, which was designed specifically to determine the presence of faecal coliforms in ice cube samples, acquires health significance. It is indicated that free residual chlorine concentration in water is indicative in measuring the effectiveness of chlorine as a disinfecting agent; whereby the concentration should range between 0.2-1.0 mg/L (Chesebrough, 2006; WHO, 2008). Being dependent to pH, alkaline water requires a higher concentration of free residual chlorine for adequate disinfection i.e. 0.4-0.5 mg/L at pH 6-8 and up to 0.6 mg/L at pH 8-9, while above pH 9 chlorination of water may not be effective (WHO, 2008). Although turbidity of less than 5 NTU for drinking water has been regarded as acceptable (Food Act 1983 (Act 281) & Regulations, 2004; Cheesebrough, 2006; WHO, 2008), ideally the median turbidity should be lower than 0.1 NTU for effective disinfection (WHO, 2008). In the context of this study, it is pertinent to indicate that in Malaysia similar microbiological and physicochemical standards are not currently available for ice cubes, thus for the purpose of discussion such standards established for drinking water are used in indicating faecal contamination and levels of physicochemical parameters in ice cubes.

Despite the fact that analysis on ice cubes for microbial contamination has not been included in the routine inspection of food outlets by the District Health Office (Zaliha & Abdullah, 2004), our results revealed that ice cubes could be a potential source for harmful microbial infection leading to food and water poisonings. In this preliminary study, contamination of ice cubes by faecal coliforms was evident in 16 (53%) out of 30 food outlets surveyed and in 3 food (10%) outlets the faecal coliform counts exceeded that of 50 CFU/100 mL, which can be construed as grossly polluted (Cheesebrough, 2006). Therefore, it appears that inference drawn on the status of cleanliness of food outlets without considering microbial analysis of ice cubes may be misleading and has tremendous health implications in the community. Since contamination by faecal coliforms was not detected in tap water as well as in the commercially bottled drinking water, it can be implied that (1) contamination by faecal coliforms may occur due to improper handling of ice cubes at the food outlets or (2) they may not be the water sources used for making the ice cubes. Being similar in the physicochemical properties with the ice cube samples found contaminated with faecal coliforms, the absence of faecal coliforms in ice cube samples in the remaining 14 (47%) food outlets may be explained by proper handling and/or storage practices of ice cubes by food operators.

Moreover, in all samples the free residual chlorine concentrations (0.01-0.07 mg/L) were found to be insufficient in rendering effective disinfection against microbial pathogens since the observed pH values ranged between 5.5 and 7.3. As prescribed by the WHO (2008), for such a range of pH the appropriate concentration of free residual chlorine should be within the range of 0.4-0.5 mg/L for rendering effective disinfecting activity. Due to the fact that the optimum pH range for coliforms to grow is between 6.0 to 7.0 (Hernandez-Delgado & Toranzos, 1995) and since the concentrations of free residual chlorine in all ice cube samples were insufficient to render effective disinfection, it was possible that ineffective disinfection of water used in making the ice cubes may lead to such high counts of faecal coliforms observed in the 16 (53%) food outlets included in this study. In addition, turbidity values in all ice cube samples (0.14-1.76 NTU) as well as in tap water (3.02 NTU) and commercially bottled drinking water (0.13 NTU) were within the acceptable range of <5 NTU (Food Act 1983 (Act 281) & Regulations, 2004; Cheesebrough, 2006; WHO, 2008).

We conclude that contamination by faecal coliforms in ice cubes among food outlets in Kubang Kerian, Kelantan may have significant health implication to the community at large. Therefore, we suggest that analysis of ice cubes for microbial contamination to be included in the routine check for hygienic standard of food premises and microbiological quality of food conducted by the local health authority. In this context, the establishment of the national microbiological guideline for ice cubes may prove useful. Although ice cubes at the food outlets were sampled and analyzed for contamination by faecal coliforms; the same was not done on ice cubes at the production, packaging and distributing levels as well as on the handling and storing practices of ice cubes at the respective food outlets. Such studies would prove valuable in indicating the point of contamination by faecal coliforms in ice cubes.

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