Assessment of microbial quality of ice and source water used for preservation of fish quality in the southern province of Sri Lanka

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Abstract The present study assessed the microbial quality of manufactured ice and the source water used for commercial fish post-harvest handling in the Southern Province of Sri Lanka. Source water and ice samples were collected from 15 ice plants between October 2021 and October 2022 and immediately analyzed for aerobic plate count (APC) at 37^0C, total coliforms, faecal coliforms, *Escherichia coli* (*E. coli*) and *Salmonella* species using SLSI protocols. The information on workplace sanitation was obtained through a questionnaire survey. Source water and ice contained APC in the range of $6.2x10^1$ - $6.9x10^4$ and $4.1x10^1$ - $1.9x10^4$ CFU/mL, respectively. Sixty-seven per cent of ice samples and sixty per cent of water samples collected exceeded the SLS limit for APC (100 CFU/mL). Faecal coliform counts in source water and ice ranged between 4 and 783 MPN/100 mL and 4 and 242 MPN/100 mL, respectively. Ninety three percent of source water and ice samples were contaminated with *E. coli*, and the counts varied between 0 and 145 MPN/100 mL and 0 and 105 MPN/100 mL, respectively. No *Salmonella* contamination was observed in water or ice samples. Of the three districts in the province, Matara showed the lowest count for the tested parameters. Source water used for ice production was a mixture of different water sources. Results revealed that the workplace sanitary conditions were significantly correlated with the microbial quality of ice ($p<0.05$). An urgent need is required to improve the sanitary conditions in ice plants of the Southern province to avoid faecal bacteria contamination in the production line of ice plants from source water extraction. Implementing sanitary standards and operational procedures in manufacturing plants is also recommended.

Keywords: Coliform bacteria, *E. coli*, fish safety, ice plants, *Salmonella* spp., sanitary conditions

INTRODUCTION

Ice is commonly used to preserve the freshness of various edible items, including fish and fishery products (Gerokomou, *et al.*, 2011) by reducing the temperature and thereby retarding the growth of microorganisms (Anupama *et al.*, 2021). Fish and fish products can get contaminated with pathogenic and spoilage microorganisms at any point throughout the production and supply chain. As such, properly preserved fish with good quality ice ensures fish food safety and provides high revenue for fishers even after long fishing trips. Fish can also be stored in good quality ice for a few days before selling to overcome price reductions at the market during glut landings (Mako, *et al.*, 2014). Since ice is used in large quantities to preserve the quality of fish, the production of clean and safe ice is of utmost importance. In most cases, many enterprises manufacturing perishable products use their own ice-making machines to produce ice for their production process (Wills, 2016).

Southern Province is one of the leading food fish supply locations in Sri Lanka. According to the Statistics Unit in the Ministry of Fisheries and Aquatic Resources Development (MFARD, 2021), 34 activated ice plants (nine in Galle, 11 in Matara and 14 in Hambantota districts) were in the Southern Province in the year 2020. The daily production capacities in the ice plants in Galle, Matara and Hambantota, were 289, 397 and 600 Mt/day, respectively, in the same year. Southern Province produced the highest amount of ice compared to the other provinces (MFARD, 2021). However, recent observations made by government officials and consumer complaints about low-quality fish indicate microbial contamination and poor handling practices along fish supply chains (Ariyawansa *et al.*, 2016).

Microbial contamination of fish reflects the use of uncleaned utensils, contaminated water and ice, inadequate ice, and unhygienic handling practices (Saritha, 2014). The ability of ice to be a vehicle for transmission of pathogenic microorganisms has been extensively studied in different geographical regions and documented (Dickens *et al.*, 1985; Daniels *et al.*, 2000; Brett *et al.*, 2014). As such, evaluating microbial contamination of ice is essential to verify potential health risks to consumers, especially raw meat consumers (Dickens *et al.*, 1985). Microbial contamination of source water is also essential since it may directly affect the quality of produced ice. Since there is no published scientific information on microbial contamination of ice and source water in the Sri Lankan context, the present study fills this gap by assessing the microbial quality of produced ice and the source water used primarily for commercial fish post-harvest handling in the Southern Province of Sri Lanka.

METHODOLOGY

Study area and sample collection

The study area was the Southern Province of Sri Lanka. The sampling locations were the selected ice plants from three districts: Galle, Matara and Hambantota. Five ice plants from each district (G1-G5 from Galle, H1-H5 from Hambantota and M1-M5 from Matara) were selected using a simple random sampling approach. All the ice plants selected were independent in operation. Source water (municipal water, dug well water, tube-well water and seawater) and crushed block ice were the sample types for the present study. The study was carried out from October 2021 to October 2022. Water samples were aseptically collected from the water inlet of ice-making machines at 15 ice plants. For each plant, triplicate samples were taken using 1,000 mL sterile containers. Similarly, triplicate ice samples, each weighing 1,000 g were directly obtained from the crushed block ice-making machines of the ice plants and sealed in sterile polyethylene bags. All the samples collected were stored in ice in chill bins at 4^0C. All sample units in chill storage were transported to the Quality Control Laboratory of the Institute of Post Harvest Technology at the National Aquatic Resources Research and Development Agency (NARA) within two hours to be analyzed.

Microbial analysis of water and ice samples

After confirming the autoclaved agar and utensils were free from bacteria, water and manufactured ice samples were tested for Aerobic Plate Count (APC) at 37^0C, total coliforms, faecal coliforms and *E. coli*. APC was tested using the pour plate technique on plate count agar (SLS 516: part 1:2013). Coliforms, faecal coliforms and *E. coli* counts were done using the MPN technique (SLS 1461 Part 1:2013). Four successive stages were done to determine whether *Salmonella* spp. was present in the water sample according to SLS 516 Part 5:2017. However, one replicate ice sample from each plant was checked for *Salmonella* spp. due to some practical difficulties.

Questionnaire survey on sanitary conditions of the ice plants

A questionnaire survey was carried out based on the methodology given by Tantrakarnapa *et al.*, (2010) and the manual on recommendations and methods for producing and handling ice with care to prevent microbiological contaminations issued by the National Aquatic Resources Research and Development Agency (NARA), (2019). Relevant information on workplace sanitary conditions was collected from the key person (head of the operational phase) of the ice plant and three workers using face-to-face interviews and questionnaires. In addition, workplace visits were also made to determine the ground conditions. One interviewer was involved in the questionnaire survey to get unbiased information. The survey comprises six requirements concerning location and manufacturing buildings, tools, machinery and production equipment, control of production processes, sanitation (sanitary facilities), cleaning and maintenance, and personal hygiene of staff. All survey requirements were classified into five types, and were scored by applying the collected information as "0 - Poor, 1 - Fair, 2 - Good, 3 - Very good, 4 – Excellent".

Statistical analysis

Since the data indicated a non-normal distribution, the Wilcoxon test was performed using the Minitab - 17 statistical software package to see whether there were significant differences among selected microbial parameters of the source water sample and the ice sample. The correlation between ice plant sanitary conditions (in terms of percentage of total scores) and microbial quality of ice (in terms of Aerobic Place Count) was

analyzed using Spearman's rank correlation at a significance level of 0.05 since the variables were not normally distributed. The correlation between source water and manufactured ice was also analyzed.

RESULTS AND DISCUSSION

The Aerobic Plate Count (APC), and Coliform bacteria counts (total coliforms, faecal coliforms and *E. coli*) in Hambantota, Matara and Galle are given in Table 1, Table 2 and Table 3 respectively.

Aerobic plate count

The Aerobic Plate Count (APC) is used as an indicator of bacterial populations of a sample. APC in ice of ice plant G3 (in Galle district) was higher than that of the respective source water. Nevertheless, all the ice plants in the Hambantota district showed significantly higher APC than the source water in the ice manufacturing process. The APC of both ice and source water exceeded the SLS standard (<100 CFU/mL). However, the APC of source water and manufactured ice of all five ice plants in the Matara district complied with the SLS standard (<100 CFU/mL). Eight out of fifteen ice plants showed a significant difference in APC between ice and water samples, with most exhibiting an increase in APC numbers in the ice samples.

Coliform bacteria count

The coliform bacteria in source water used in H1 and G5 ice plants showed significantly high values($p<0.05$) and when producing ice, the H3 plant also showed high coliform counts. This observation could be attributed to the poor sanitation conditions in these plants during the ice manufacturing process. *E. coli* was not detected in water samples collected from the G4 ice plant. Nevertheless, in many ice plants, the coliform counts in ice were lower than in the respective source water. According to Bai *et al.* (2022) the coliform bacteria proliferate at $>35^0$C. Hence, at low temperatures, the coliform bacterial count is expected to be low. Although the total coliform in respective ice samples was comparatively low, all samples did not comply with the SLS standard. A similar pattern was also observed for the faecal coliform counts in the three districts. *E. coli* of all samples in the three districts except G4 at Galle district did not comply with the SLS standards. Nevertheless, all samples tested were negative for *Salmonella* spp.

Percentage compliance of microbial counts of ice and source water with SLS standards are given in Figure 1 and Figure 2, respectively. Accordingly, 100 % of the water and ice samples failed to meet the national standards of Sri Lanka for faecal coliform parameters. Ninety three per cent (93%) of the water and ice samples failed to meet the national standards of Sri Lanka for total coliform and *E. coli* parameters. Also, 60% of the water samples and 67% of the ice samples failed to meet the national standards of Sri Lanka for APC parameters.

Health risks associated with E. coli contaminated ice

Health risk levels of *E. coli* in ice samples (based on WHO guidelines) are given in Figure 2. Of the 15 ice plants, 6% had *E. coli* below 1MPN/100 mL, indicating no health risk, while 67% of samples had *E. coli* between 1-10 MPN/100 mL, indicating low health risk. However, 27% of samples showed moderate to high health risk due to the contamination of *E. coli*. This contamination could highlight that using *E. coli-contaminated* ice in fish preservation may pose health risks, especially for raw meat consumers.

Source water of ice plants

The source water used for ice production varied among the ice plants (Table 4). Ice plants that used dug well water and treated municipal water of G5 plant showed the highest *E. coli* count. This may be attributed to the poor treatment of municipal water and the saltwater intrusion. The shallow water table and high permeable soil in the coastal areas could also be a key reason for microbial contamination of dug well water (Indika *et al.*, 2022).

Table 1. Mean bacterial count in water and ice detected in sampling stations (H1-H5) of Hambantota district between October 2021 and October 2022. Data are presented as mean ± SEM. Mean values indicated by different superscript letters between water and ice of each plant are significantly different from each other (Wilcoxon rank test, $p<0.05$).

Bacteria	Mean bacterial count per sampling station										SL Standards for water and ice quality suitable for human consumption
	H1		H2		H3		H4		H5		
	Water	Ice	Water	Ice	Water	Ice	Water	Ice	Water	Ice	
Total coliform (MPN/100mL)	$1867^{a}\pm 11$	$1600^{b}\pm 00$	$65^{a}\pm 02$	$23^{a}\pm 03$	$112^{a}\pm 18$	$692^{b}\pm 61$	$100^{a}\pm 46$	$27^{a}\pm 08$	$38^{a}\pm 12$	$46^{a}\pm 08$	<10
Faecal coliform(MPN/100mL)	$783^{a}\pm 20$	$242^{b}\pm 29$	$13^{a}\pm 08$	$15^{a}\pm 05$	$25^{a}\pm 11$	$18^{a}\pm 03$	$16^{a}\pm 05$	$10^{a}\pm 04$	$28^{a}\pm 10$	$42^{a}\pm 03$	Null
E. coli (MPN/100mL)	$50^{a}\pm 28$	$12^{b}\pm 12$	$08^{a}\pm 04$	$03^{a}\pm 02$	$21^{a}\pm 04$	$105^{a}\pm 61$	$10^{a}\pm 04$	$06^{a}\pm 03$	$26^{a}\pm 10$	$26^{a}\pm 13$	Null
APC(cfu/mL)	$6966^{a}\pm 11$	$15400^{b}\pm 12$	$3378^{a}\pm 51$	$4032^{a}\pm 75$	$667^{a}\pm 28$	$6000^{b}\pm 40$	$3848^{a}\pm 44$	$3500^{a}\pm 12$	$627^{a}\pm 24$	$833^{a}\pm 54$	100
Salmonella spp.	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 2. Mean bacterial count in water and ice detected in sampling stations (M1-M5) of Matara district between October 2021 and October 2022. Data are presented as mean ± SEM. Mean values indicated by different superscript letters between water and ice of each plant are significantly different from each other (Wilcoxon rank test, $p<0.05$).

Bacteria	Mean bacterial count per sampling station										SL Standards for water and ice quality suitable for human consumption
	M1		M2		M3		M4		M5		
	Water	Ice	Water	Ice	Water	Ice	Water	Ice	Water	Ice	
Total coliform(MPN/100mL)	$63^{a}\pm21$	$47^{a}\pm29$	$68^{a}\pm36$	$42^{a}\pm08$	$75^{a}\pm53$	$11^{a}\pm05$	$16^{a}\pm02$	$26^{a}\pm02$	$24^{a}\pm07$	$15^{a}\pm06$	<10
Fecal coliform(MPN/100mL)	$45^{a}\pm31$	$14^{a}\pm09$	$16^{a}\pm06$	$14^{a}\pm05$	$19^{a}\pm02$	$07^{b}\pm01$	$11^{a}\pm04$	$10^{a}\pm01$	$15^{a}\pm03$	$08^{b}\pm03$	Null
E. coli (MPN/100mL)	$6^{a}\pm1$	$6^{a}\pm1$	$4^{a}\pm2$	$5^{a}\pm4$	$2^{a}\pm2$	$1^{a}\pm1$	$4^{a}\pm3$	$4^{a}\pm0$	$5^{a}\pm3$	$3^{a}\pm1$	Null
APC (cfu/mL)	$64.83^{a}\pm12.55$	$57.00^{a}\pm22.06$	$98.00^{a}\pm9.73$	$96.0^{a}\pm34.03$	$62.50^{a}\pm14.08$	$41.50^{a}\pm12.12$	$91.17^{a}\pm7.69$	$98.83^{a}\pm13.77$	$64.83^{a}\pm4.65$	$54.17^{a}\pm9.93$	100
Salmonella spp.	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 3. Mean bacterial count in water and ice detected in sampling stations (G1-G5) of Gall district between October 2021 and October 2022. Data are presented as mean ± SEM. Mean values indicated by different superscript letters between water and ice of each plant are significantly different from each other (Wilcoxon rank test, $p<0.05$).

Bacteria	Mean bacterial count per sampling station										SL Standards for water and ice quality suitable for human consumption/ 100mL)
	G1		G2		G3		G4		G5		
	Water	Ice	Water	Ice	Water	Ice	Water	Ice	Water	Ice	
Total coliform(MPN/100mL)	$23^{a}\pm10$	$17^{a}\pm05$	$55^{a}\pm48$	$18^{a}\pm09$	$45^{a}\pm22$	$28^{a}\pm06$	$10^{a}\pm03$	$08^{a}\pm01$	$1666^{a}\pm 11$	$375^{b}\pm16$	<10
Fecal coliform(MPN/100mL)	$9^{a}\pm3$	$12^{a}\pm5$	$13^{a}\pm8$	$5^{a}\pm5$	$24^{a}\pm10$	$9^{a}\pm2$	$4^{a}\pm2$	$4^{a}\pm2$	$232^{a}\pm 10$	$32^{b}\pm21$	Null
E. coli (MPN/100mL)	$6^{a}\pm2$	$7^{a}\pm2$	$8^{a}\pm4$	$2^{b}\pm2$	$6^{a}\pm1$	$6^{a}\pm1$	$0^{a}\pm0$	$0^{a}\pm0$	$145^{a}\pm 12$	$12^{b}\pm6$	Null
APC (cfu/mL)	$677^{a}\pm 29$	$677^{a}\pm 24$	$103.83^{a}\pm 13.87$	$4412^{b}\pm 12$	$63.50^{a}\pm 9.73$	$15867^{b}\pm 13$	$25^{a}\pm 39$	$114.3^{b}\pm 32.42$	$61.07^{a}\pm 22.86$	$66.00^{a}\pm 12.56$	100
Salmonella spp.	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

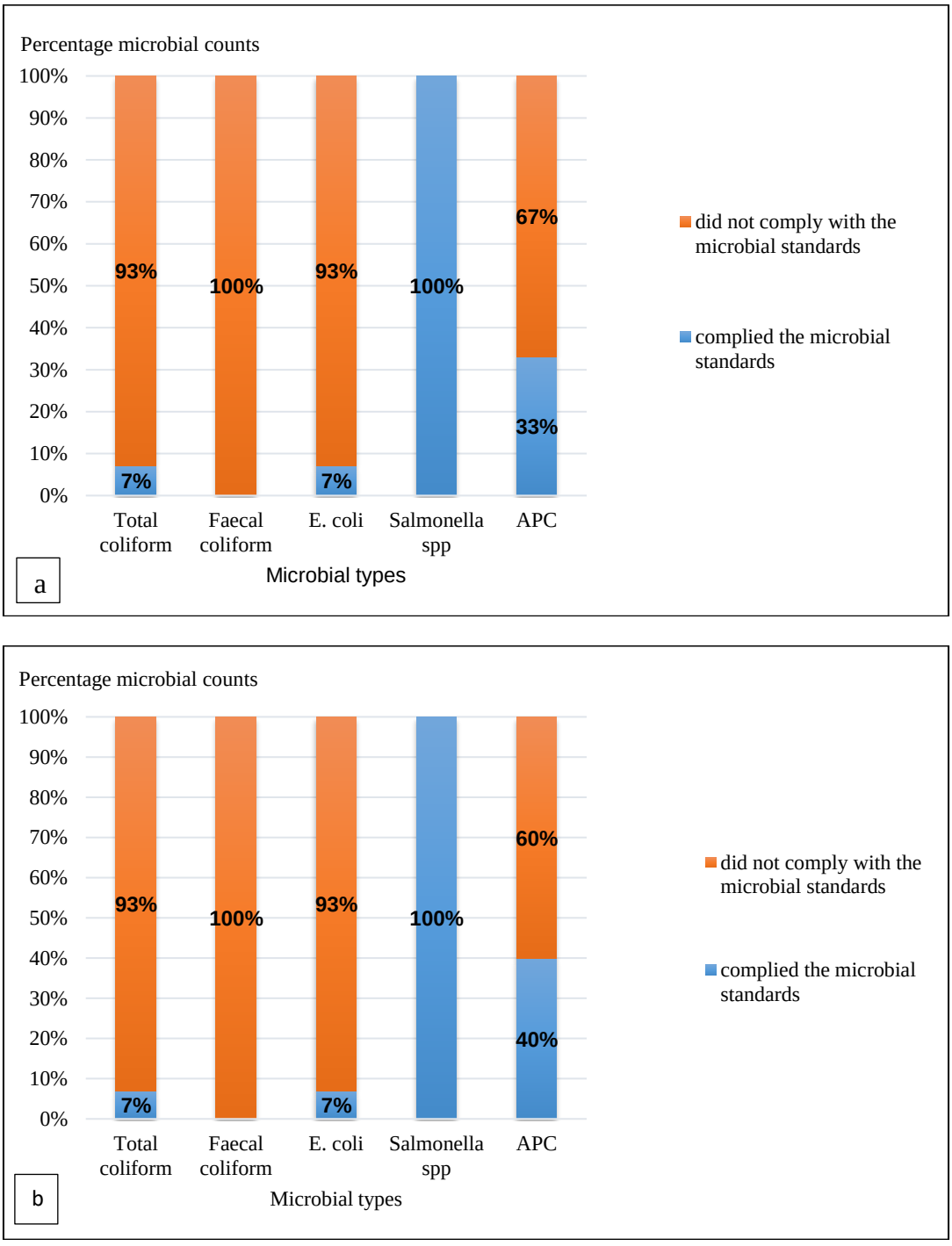

Fig 1. Compliance of microbial counts in ice (a) and source water (b) with SL Standards for water and ice quality suitable for human consumption

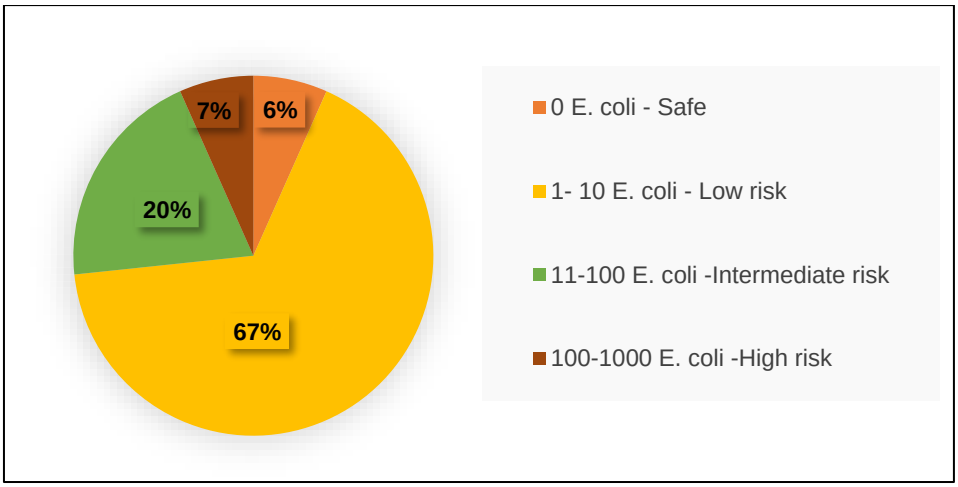

Fig 2. Risk level of *Escherichia coli* (CFU/100 mL) in ice samples based on WHO guidelines

Table 4. *Escherichia coli* levels in ice and water, and the source of water detected in ice plants from Hambantota (H1-H5), Matara (M1-M5) and Galle (G1-G5) districts

Ice plant	Source	*E. coli* in water (MPN/100mL)	*E. coli* in ice (MPN/100mL)
H1	dug well and tube well	50±3	32±1
H2	dug well and treated municipal water	8±4	3±2
H3	seawater and treated municipal water	21±4	105±6
H4	dug well and treated municipal water	10±4	6±3
H5	dug well and tube well	26±1	26±1
M1	dug well and tube well	6±1	6±1
M2	dug well and tube well	4±2	5±4
M3	dug well and treated municipal water	2±2	1±1
M4	dug well and tube well	4±3	4±0
M5	dug well and treated municipal water	5±3	3±1
G1	dug well, tube well and treated municipal water	6±2	7±2
G2	dug well and tube well	8±4	2±2
G3	dug well, tube well and treated municipal water	6±1	6±1
G4	tube well and treated municipal water	0±0	0±0
G5	dug well and treated municipal water	145±12	12±6

Workplace sanitation

Sanitary management practices in ice plants were evaluated (Table 5) with scores ranging from 16 to 76 out of 128. Data analysis revealed significant variations and plants with higher scores showed the best sanitary conditions and management, while H1 and G3 had the lowest scores due to poor sanitary conditions. Plants in Matara district and G4 and G5 in Galle scored above 53, indicating relatively better structural facilities.

Despite food safety certifications, most plants showed poor maintenance and monitoring. Regular water quality checks were lacking, and filtering systems were not consistently functional. Field inspections further revealed inadequate premises maintenance, with unclean floors and equipment. Improper manual handling of ice and poor employee hygiene practices contributed to high microbial contamination.

Annual employee health checkups were rare, and temporary employment contributed to inconsistent hygiene standards. Poor personal hygiene and lack of production monitoring compromised worker safety, while equipment without proper maintenance and unsanitary handling affected ice quality. No plant scored the highest (4) in any sanitation category, highlighting the need for improved sanitation management, equipment upgrades, and stricter adherence to food safety regulations.

Table 5. Allocation of marks for the sanitary conditions and better management practices carried out in ice plants (0- Poor, 1- Fair, 2- Good, 3- Very good, 4- Excellent)

Sanitary conditions, better management practices and their subcategories used during the questionnaire survey and observation	Identification **number of ice plants and allocated marks**														
	H1	**H2**	**H3**	**H4**	**H5**	**M1**	**M2**	**M3**	**M4**	**M5**	**G1**	**G2**	**G3**	**G4**	**G5**
Location and manufacturing buildings															
Clean environment	1	1	1	1	1	3	2	3	2	3	1	1	1	2	3
Separate rooms for each task	2	1	1	1	1	2	1	2	1	2	1	1	0	2	2
Availability of water quality testing laboratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tools, machinery and production equipment															
Utensil available for scooping the ice	2	1	1	0	1	3	2	3	2	1	0	0	0	1	1
The ice is always removed from the bucket/ice machine using a clean utensil such as a scoop	0	1	1	0	1	2	2	2	2	2	0	0	1	2	2
Equipment is checked throughout the day for any sign of contamination	0	1	1	0	1	3	2	2	2	2	1	1	1	2	3
Equipment is not corroded	1	0	1	1	0	2	2	2	1	3	1	1	0	1	3
Control of the production process															
Public water supply is used	1	1	1	1	0	2	2	2	1	1	0	0	0	1	2
Water sources used for the ice production is a municipality water line	1	1	1	1	0	3	1	2	2	2	0	1	1	1	2
Type of water source is freshwater	0	1	1	1	1	3	3	3	2	3	1	1	0	1	3
Regular monitoring of water quality	0	0	1	0	1	2	3	3	2	3	1	0	0	2	3
Bottom siphoning of the storage tank	0	1	1	0	1	3	2	2	3	2	0	0	1	1	2
Use of a good water filtration system for the intake of water	1	1	1	1	0	2	2	2	1	2	1	1	1	1	2
Sanitation (Sanitary facilities)															
Water treatment methods for intake of water	0	1	0	1	0	2	1	2	1	2	1	1	1	2	3
Store ice in a cool room immediately prior to use	0	0	1	0	1	2	2	2	2	3	1	1	1	1	2
Cover ice machine	1	0	1	0	0	2	2	2	2	3	0	1	0	1	2
Public has access to the ice buckets/ice machine	1	1	1	1	0	2	3	3	2	1	0	0	0	1	2

Ice storage bins and ice buckets are always kept covered	0	1	1	1	1	3	1	3	2	2	1	1	0	2	3
No pests, or animals present on the premises	1	1	0	1	1	3	1	3	1	2	1	1	0	2	3
Use safety method to move ice blocks from storage to crushing machine	1	1	1	1	1	3	1	2	2	2	1	0	1	3	2
Disinfection of equipment	0	1	1	1	0	2	2	2	2	3	0	1	0	2	1
Ice machine is not connected to the main water supply	0	0	1	1	0	3	2	2	2	3	0	0	0	2	1
Cleaning and maintenance															
Staff is following manufacturers' instructions in the use of ice-making machines	1	1	1	0	1	2	2	3	1	3	1	1	1	1	2
Melted ice is not present with ice	0	1	0	1	1	3	2	3	2	2	0	1	1	2	3
Ice manufacturing area and storage compartment are cleaned regularly	0	1	1	1	1	3	2	3	2	3	1	1	0	2	2
Scoops and other utensils in contact with the ice are cleaned at the end of every working day	0	1	1	1	0	2	3	3	1	2	1	1	1	2	2
Check the water quality used to make ice	0	0	1	1	2	2	2	2	2	2	1	0	0	3	2
Personnel and hygiene workers															
The person dispensing ice from the machine or the storage bin wash and dry their hands thoroughly before starting the task	0	1	1	0	0	2	1	3	1	1	0	1	1	2	2
Regularly maintenance of workers' hygiene	0	1	1	1	1	2	1	2	2	2	1	0	0	2	3
Follow the health monitoring regularly	0	1	0	1	0	3	2	2	2	2	1	1	0	2	3
Wear separate gloves, hair caps and boots	1	1	0	1	0	3	2	2	2	3	1	0	1	2	2
Having skills to detect bad hygiene conditions	1	2	1	1	1	2	2	2	1	3	0	1	2	2	3
Total Marks	**16**	**26**	**26**	**22**	**19**	**76**	**58**	**74**	**53**	**70**	**19**	**20**	**16**	**53**	**71**

The correlation between APC and ice plant sanitary conditions

The correlation between the microbial quality of ice (in terms of Aerobic Place Count) and ice plant sanitary conditions (in terms of percentage of total scores) was analyzed by Spearman's rank correlation at a significance level of 0.05. The Spearman's correlation coefficient (ρ) value (-0.934) indicated a strong significantly negative ($p<0.05$**)** monotonic relationship between ice plant sanitary conditions and microbial quality of ice. When the microbial count of ice increases, ice plant sanitary conditions tend to decrease.

The correlation between APC in water and APC in ice

The correlation between the microbial quality of ice and microbial quality of water (in terms of Aerobic Place Count) was also analyzed using Spearman's rank correlation test at a 0.05 significance level. Spearman's correlation coefficient (ρ) value (0.499) indicated a non-significant ($p>0.05$) moderate positive correlation between the microbial quality of ice and water.

Conclusions and recommendations

The APC of both ice and source water in ice plants selected for the study from the Hambantota and Galle districts exceeded the SLS standard (<100 CFU/mL). The study further revealed that workplace sanitary conditions were significantly correlated with the microbial quality of ice. No *Salmonella* contamination was observed in water or ice samples. Of the three districts in the province, Matara showed the lowest count for the tested parameters. An urgent need is required to improve the sanitary conditions in ice plants of the Southern Province to avoid faecal bacteria contamination in the production line of ice plants from source water extraction. Implementing sanitary standards and operational procedures in manufacturing plants is also recommended.

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