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Pathogenesis and Toxins

Physical, chemical and microbiological quality of ice used to cool drinks and foods in Greece and its public health implications

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ABSTRACT

Ice used for direct human consumption or to preserve foods and cool down drinks can be contaminated with pathogenic microorganisms and may potentially become a vehicle for consumer's infection. To evaluate physical, chemical and microbiological quality of commercial ice and ice used for fish and seafood, 100 ice samples collected at 10 different retail points in the region of Epirus were studied. The following microbiological parameters were determined: Total coliforms, fecal coliforms, *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Escherichia coli*, *Campylobacter* sp., *Vibrio cholerae*, *Aeromonas* spp., *Pseudomonas aeruginosa* and *Clostridium perfringens*.

E. coli was detected in 22% and coliforms were detected in 31% of samples. Samples in which coliforms were detected fail to meet the microbiological criteria specified by the drinking water legislation.

Aeromonas spp., *Shigella* spp., *Campylobacter* sp. and *V. cholerae* were not detected. Spore forms of *C. perfringens* were prevalent at 35% and the psychotropic bacterium's *P. aeruginosa* and *Yersinia* spp. were found only at three samples each.

The presence of large numbers of coliforms as well as of other pathogenic strains suggested that commercial ice and ice used to make cool drinks or in preservation of fish and seafood may represent a potential hazard to the consumer. In view of the results reported herein, it is highly recommended that national regulatory guidelines should be established for the production of ice as long as regular inspections.

1. Introduction

Epirus (north-western Greece) has an extended coastline in which many fisheries flourish. There is also an important professional fleet of vessels fishing in the open Ionian and Adriatica seas. A large quantity of the products of these activities is absorbed from the local markets for retail commerce and for the numerous hotels and restaurants of the area. Throughout this procedure raw fish is maintained in ice. Furthermore, ice is used in large quantities to cool drinks and refreshments, given that the area is a very popular tourist destination during spring and summer. According to the European legislation, ice oriented for foods should be prepared from potable water but often, due to the poor water quality or due to handling and transport practices, it becomes a vendor of pathogens causing outbreaks of gastroenderitis [1–3].

The objective of this study was to investigate the microbiological quality of the ice used in Epirus area in an effort to monitor the hygienic status of commercial ice and draw conclusions on its possible impact to public health.

2. Material and methods

One hundred ice samples weighting 5 Kg, collected at 10 deferent retail points in the region of Epirus were evaluated. Sampling took place during the first five months of 2009. Ice samples were collected in sterile specimen cups that were sealed in resealable zipper storage bags and transported to the laboratory for analysis. Time between collection and analysis was

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approximately 2 h to allow time for the ice to melt before plating samples. Serial dilutions of the ice were prepared using 0.1% sterile peptone.

Total aerobic microorganisms were determined on ice samples by plating 0.1 mL of the serial dilutions on plate count agar (Becton Dickinson, Sparks, MD). Petri dishes containing plate count agar and samples were incubated at 35 °C for 48 h.

Each ice sample was analyzed for *C. perfringens*, *Enterococcus* sp., fecal coliforms, *E. coli*, *Salmonella* sp., *Pseudomonas* sp., *Campylobacter* sp., *Yersinia* sp., *Vibrio* sp., *Shigella* spp. and *Aeromonas* spp.

Samples (100 mL) were diluted 1:10 and analyses were performed using the membrane filtration technique as described in the standard methods proposed by the American Public Health Association -APHA, 1992 [4]. The following growth media were used: m-Endo agar (Difco, Detroit, MI), mFC agar, (Difco, Detroit, MI), Slanetz and Bartley agar (Oxoid, Basingstoke Hampshire, UK).

For Campylobacter, filtration method was used: Pre-enrichment was done, followed by 500-mL sample filtration. Preston broth was incubated at 37 °C for 4 h, followed by incubation at 42.5 °C for 20 h under microaerophilic conditions (5% O_2, 85% N_2, 10% CO_2).

For *C. perfringens,* a 100-mL quantity of the initial sample was filtered through a sterile membrane with a porosity 0.45 μm, which retained the microorganism. This last membrane filter was placed in a tube with 9 mL of L.S. broth. The composition of the lactose sulfite broth (LS broth) [5] was as follows: 5 g of tryptic digest of casein, 2.5 g of yeast extract (Difco, Detroit, MI), 2.5 g of sodium chloride, 2.5 g of lactose, 0.3 g of L-Cys hydrochloride, 1 L of distilled water. The pH was to adjusted 7.1 ± 0.1, and 9 mL of the medium was dispensed into tubes. Sterilization was performed by autoclaving at 115 °C for 20 min. Before use, the medium was boiled for 20 min to reduce the oxygen content and 0.5 mL of a 1.2% solution of anhydrous sodium metabisulfite ($Na_2S_2O_2$) and 0.2 mL of a 1% solution of ferric ammonium citrate were added to each tube. The above solutions were prepared and sterilized by filtration (0.45 μm) just prior to use. The medium was shaken, and from this tube (10^{-1}) two further dilution steps up to 10^{-3} were made. Incubation was performed aerobically in a water bath at 46 °C for 24 h. An aliquot of each sample was heated for 20 min at 80 °C for detection of germinated spore forms, and for each an LS broth tube was seeded.

Standard procedures were performed for the identification of aerobic microflora to the species level. Finally, ice samples were analyzed for the presence of *Shigella*, *Aeromonas* spp, *P. aeruginosa* and *Yersinia* spp. by methods as described in the standard methods [6].

In the *V. cholerae* assay, one liter of water was placed in alkaline peptone water, pH 8.4, and incubated at 37 °C for 6 h. Isolates were obtained from the surface pellicle of the enrichment broth on DIFCO Thiosulfate-citrate-bile-salts-sucrose agar (TCBS) and incubated at 37 °C for 24–48 h. Biochemical and serological identification of suspected colonies were performed [7].

All samples were analyzed in duplicate.

2.1. Chemical analyses

Ice samples were evaluated for pH, Turbidity (N.T.U.) [total suspended solids (TSS)], Alkalinity in mg/L $CaCO_3$, Chlorine in mg/L Cl- and Nitric salts in mg/L. The pH was measured directly on melted ice using a Ph meter (Fisher Scientific Accumen Model 10 pH meter, Pittsburgh, PA).

The turbidity reading was then recorded using (HACH RATIO/XR) instrument, this was done by taking the lowest reading [4]. Also the other chemicals analyses were performed by using standard methods [4].

3. Results

3.1. Microbiological analysis

Our results are shown in Table 1. *Shigella* spp, *V. cholerae* and *Aeromonas* spp were not isolated. Total coliforms were isolated from 37 samples (37%), while fecal coliforms from 25%. Total coliforms in positive samples ranged from 1 to 97 cfu/100 ml and fecal coliforms from 1 to 100 cfu/100 ml. *E. coli* was found in 15% of the samples and from 1 to 50 cfu/100 ml. *Salmonella* spp, *P. aeroginosa* and *Yersinia* spp were isolated from 4% (1–55 cfu/100 ml), 3% (1–22 cfu/100 ml) and 2% (27 and 35 cfu/100 ml) of the samples respectively. Finally vegetative forms of *C. perfringens* were isolated from 18% of the samples while the spore forms from 33% of the samples.

3.2. Chemical analysis

The results of the chemical analyses are presented in Table 2. pH values ranged from 7.2 to 8.3 with a mean value of 7.7. Mean Turbidity was 1.6 N.T.U. Most of the values were below 0.5 units but 13 samples had values greater than 2 and up to 13 N.T.U. Alkalinity ranged from 24 to 140 mg/L with an average of 56.5 mg/L. Chlorides (as mg/L Cl^-) varied between 5 and 110 mg/L but most of the samples (93%) were below 10 mg/L. Nitrates concentration ranged from below 1–75 mg/L (mean = 10.7). Six of the nitrates values exceeded the guideline of 50 mg/L.

4. Discussion

Ice used to cool drinks and refreshments, to maintain raw fish, and to other culinary purposes, can be a vehicle for various pathogens [1,2,8]. Especially in areas with hot or temperate climate this danger may impose a serious threat to the consumers [2,9]. The objective of this study was to investigate the microbiological quality of the ice used in Epirus area (North western Greece) in an effort to monitor the hygienic status of commercial ice and draw conclusions on its possible impact to public health [10,11]. Contamination of ice can be attributed to contaminated initial water source or storage tanks, equipment, packaging, storage and handling of the ice [12]. In addition to these factors, Burnett et al. (1994) [13] suggest that ice-making machines can play an important role to the contamination of ice due to seeding from the mains supply, faulty plumbing allowing backflow from the drains and irregular cleaning of the machines. The fact that in the majority of our samples pathogens were not isolated from duplicate analyses, indicates that possibly the water supply was not the main source of contamination. Staff handling on the other hand seems to be a more possible source of contamination as the low incidence of *E. coli* indicates [9]. According to Falcao et al. (1993) [14,15] the presence of fecal coliforms and *E. coli* is a strong

Table 1
Incidence of various microorganisms in 100 commercial ice samples.

Microorganisms	Positive samples (%)	Range (cfu/100 ml)
Total coliforms	37	1–95
Fecal coliforms	25	1–100
E. coli	15	1–50
Salmonella spp.	4	1–55
Yersinia spp.	2	27–35
Pseudomonas aeruginosa	3	1–22
C. perfringens (veg)	18	
C. perfringens (spore)	33	
Shigella spp.	none	
Vibrio cholerae	none	
Aeromonas spp.	none	

Table 2
Chemical analyses of 100 ice samples.

Chemical parameter (units)	Mean	Range
pH	7.7	7.2–8.3
Turbidity (N.T.U.)	1.6	0–13
Alkalinity in mg/L $CaCO_3$	56.5	25–140
Chlorides in mg/L Cl^-	20.3	5–110
Nitrates in mg/L NO_3^-	10.7	<1–75

indication of recent fecal contamination. The same reason can also explain the low percentages of *Yersinia* spp. *Salmonella* spp and *P. aeroginosa*. These bacteria share common virulence factors [16] hence implicating contaminated ice as possible vehicle in the oral-fecal route. The crew of the fishing vessels is not trained in maters of personal hygiene with respect to ice contamination and this is true for the personnel involved in the production of ice, in the retail commerce of fish and of course for the bar tenders who often handling ice with bare hands. Environmental contamination mostly airborne and through utensils, is also possible as *C. perfringens* and coliforms indicate. The ice is usually stored in open buckets or in the refrigerators beside foods of various origin, particularly in bars and restaurants, and thus is susceptible to environmental contamination [1,17]. We found no written records concerning the frequency of disinfection and cleaning of the ice-making machines and we have every reason to suspect that such practices are very infrequent. Interestingly the results of the chemical analyses do not show a specific pattern with respect to the microbiological ones. Samples with marked presence of pathogens did not present statistically different values of the chemical parameters from the other samples. In conclusion, we believe that although most of our samples were found in a satisfactory hygienic status the situation is alarming because of the many possible opportunities for contamination. Personnel involved in the production and the handling of ice should be trained in relative hygiene matters and ice-machines should be disassembled, cleaned and disinfected regularly. The regular inspection by proper authorities is also of great importance.

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