

# Contamination Of Ice And Ice Water By Vibrio Cholera In Different Regions Of Mashad, Iran

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## Abstract

**Background:** Cholera is an infective and dangerous disease that appears in endemic form in Iran and some tropical countries. It creates epidemic form in favorable conditions. The microorganism that causes this disease is vibrio cholera, which has been discovered by Robert Koch in 1883. Humans are the only natural host for this microorganism that is transmitted by eating and drinking food and beverages contaminated with feces. The disease can be controlled for a long time by drinkable water preservation and controlling food quality. Some organisms can exist in ice even for some weeks because of resistance to cold and ice hyperosmolar environments. The aim of this study is to research water and ice water for contamination by vibrio cholera because water and ice water are very needed by most people.

**Methods:** 100 samples of ice water and 100 ice in four regions of Mashhad city were evaluated by culturing in T.C.B.S media (colony on solid-agglutination test).

**Results:** The result obtained from culturing of ice and ice water show that 12% of the total samples in the initial experiment were contaminated with many vibrios. 2 cases of vibrio cholera (NAG) have been cultured (0/5). NAG (non agglutination) cholera is important because can create symptoms like cholera.

**Conclusion:** Ice and ice water may be contaminated during different stages of distribution, especially in warm seasons. Therefore, we need to prevent transmission of different organisms through water or ice.

## INTRODUCTION

In the most recent epidemic of cholera in Latin America, nearly a million cases were reported and almost 9000 people died (1) between June 1991 and December 1993. The epidemic spread rapidly. In Iran, an epidemic cholera spread throughout 1959. Inadequate sanitation facilities and use of inadequately treated water are likely responsible for the spread of the cholera.

Cholera is one of the dangerous contaminated diseases occurring in human and it has resulted in lots of mortalities in different societies in the world. The high mortality caused by the disease always made the physicians to study contagion ways in order to reducing the amount of mortality. Various pathogenic factors especially bacteria are transferred by water (2). One of the things mainly consumed in warm seasons is ice and ice water. Its contamination will lead to transmission of some organisms (3). Most of the organisms

are not able to live in ice because of its low temperature, but some of them like vibrio cholera (resistant to low temperatures) can exist in ice even up to a few weeks (4).

The aim of this study is to examine water, which is always a vital and very need for people. So we should examine the water as cause of contamination. Therefore, the objective of this study is finding the contamination of ice and ice water especially by cholera vibrio in different regions of Mashad city. Finally, we will recommend some practical and hygienic methods for ice and ice water consumption.

## MATERIALS AND METHODS

Since the main transmission way of cholera is contaminated water and food (4), we examined the water contamination by the cholera organism in cultures. Mashad city was divided to four regions: North-South-East-West. 100 ice and 100 ice water samples were taken from streets, ice factory and traveling salesmen in street border of each region. Sampling

method was random. Ice samples were carried in plastic containers (each ice sample changed to 100-200cc water after melting). Ice water samples were carried by 20cc experimental tubes. 0/2 cc sodium thiosulfate 3% was added to the collecting containers. In order to prevent microorganism from growth, the water was dechlorinated. After sampling, the ices were stored at usual temperature for 2-4 hours in order to change water. Then 2cc of total volume of each sample was taken and placed in media (10 gram sodium chloride, 10 gram pepton), usually 2cc of sample was mixed with 10cc of pepton water solution and well shaken to obtain a homogenate fluid (5,6). The samples were placed in the incubator for 6-8 hours and then cultured it in T.C.B.S (thiosulfate-citrate- bile- sucros). After 18 hours, yellow vibriion colonies appeared on the blue-green surface of the culture. The culture for cholera vibriion is special but other colonies like proteus, salmonella, antrobacter and E.coli can also grow on it. Finally, if there is any doubtful colony about the culture, an agglutination test was performed. This experiment was carried out by using polyvalan cholera antiserum.(2,3,7).

**RESULTS**

The results obtained from culturing of ice (100 cases) and ice water (100 sample) consumed by the people of Mashad showed that 12% of total samples in the initial experiment, 20 cases in ice samples and 4 in ice water samples, were contaminated by a kind of vibriion (table 1,2). Vibriion cholera type classic and Elter has not been shown in any culture of ice and ice water samples but 2 cases have been vibriion cholera N01 in ice cultured and 18 cases had vibriion non cholera (table 1). Non cholera organisms in ice water were found in 4 cases (table2). Contamination with vibriion cholera N01 can be dangerous like Eltor. Vibriious families have different mutations and about 10 human pathogens which are mainly in N01 group, have been recognized. Other cultured organisms in samples are shown (table 3). In this study 0/5% of samples have been contaminated with N01 group (table 4). Vibriion cholera N01is like vibriion cholera 01 but it cannot agglutinate with 01 antiserum. Vibriio cholera N01 is defined as NAG. In some cases, NAG agents can create symptoms like cholera such as acute and watery diarrhea and should be treated like cholera disease. NAG is usually more common at the beginning and in the end of epidemics in a region.

**Figure 1**

Table 1: Distribution of contamination ice to vibriions in different regions Mashad

Family	Genus	N0	Region
Vibriion	CholeraN01	2	*A,D
Vibriion	Non cholera	18	*A,C,D
Total	-	20	-

\*A: north      B: south      C: east      D: west

**Figure 2**

Table 2: Distribution of contamination icewater to vibriions in different regions Mashad

Family	Genus	N0	Region
Vibriion	CholeraN01	-	-
Vibriion	Non cholera	4	A,B,D
Total	-	4	-

**Figure 3**

Table 3: Distribution of samples study on bacterial culture

Microorganism	Ice	N0(Ice+Icewater)	Icewater	Percent	Region
Proteus	1	1	-	5	C
Salmonella paratyphi	9	10	1	5	B,C
Ecoli	16	26	10	13	A,B
Aerobacter	12	14	2	7	B,D
Vibriious type	20	24	4	12	A,B,D
Strill	56	125	69	62.5	A,B,C,D
Total	-	200	-	100	-

**Figure 4**

Table 4: Rate of contamination in study to vibriions and other organism

Organism	N0	Percent
Strill (negative cuture )	125	62.5
Other (Salmonella-Shigella-E.coli.....)	51	26
Vibriio non cholera	22	11
Vibriio cholera	2	0/5
Total	200	100

**DISCUSSION**

In late January 1991, epidemic cholera appeared in Peru. Within 2 months, 7922 cases and 17 deaths occurred in Piura. A study of 50 case patients and 100 matched controls demonstrated that cholera was associated with drinking unboiled water or drinking beverages from street vendors (8). In a second study, patients were more likely than controls to consume beverages with ice (8). Glass has evaluated to determine the modes of transmission of cholera in the regency of Pidie, Indonesia(8).

Cholera is an infectious disease, especially for humans. The main symptoms of the disease are caused by secreted antitoxin of vibriion in the small intestines. There is an acute diarrhea that results in water and electrolyte disorders, hypovolemic shock, metabolic acidosis and death (4,9). Through education people have become aware of this

disease and the death rate caused by the disease has decreased (5, 10). Lack of health conditions, overpopulation, food poverty, high moisture, warm weather, contaminated water and the existence of insects have main roles in spreading the disease. Agbessi traced ice produced in blocks from 10 factories in Abidjan to 10 different market places where they were sold (11). A number of factors including exposure to water and consumption of ice were associated with disease (12).

Pathogenic agents may be transmitted by direct contact with patients or via indirect ways by consuming contaminated food and beverages such as contaminated milk, vegetables, fruits, water and ice (6,13). Contaminated clothes in a moist environment can keep vibriion up to several weeks alive. Vibriion is able to exist for about 2-8 days in patients stool, up to 2 weeks in water stores, 4 days in seawater and about 4-6 weeks in ice (2,9,10). Because of the high hyperosmolarity in ice, vibriion can be alive despite surface washing resulting in transmission of the disease. 90% of the cured patients will be free of vibriion in 7-14 days and 99% in a maximum of a month. Presence of healthy carries, especially in the Eltor species up to 2 month has been reported. *Vibrio cholera* can exist in bile for along time (8,14). The specific culture medium for vibriion cholera is T.C.B.S (thiosulfate-citrate-bile-sucrose). It has two biotypes, Eltor and classic (6). Eltor grows very quickly and can be alive in water for a longer time (13). This organism can grow in alkaline environments (PH:8.5-9.5) very well, but not in acidotic environments (3,6,7,15). In droughts it becomes extinct very quickly but in moist places can exist up to one month (13,16). From the viewpoint of antigenic properties it is divided into 2 groups: vibriion cholera O1 (with antiserum agglutination) and vibriion cholera non O1 (without agglutination)(5,7). Vibriion cholera O1 has 2 biotypes: classic and eltor. Some of the vibriion cholera N01 stubs can also cause dispersed diarrhea attacks and act like type Eltor. Discovery of the first positive case of an epidemic has been found in September 1998 at the Shahid Hashemynejad Hospital of Mashhad.

### RECOMMENDATIONS

When traveling to underdeveloped countries, travelers must avoid unheated water, ice, and beverages containing ice (17). Ice that is distributed in Mashad can be contaminated during different stages of distribution, especially in warm seasons or they may be contaminated before ice production. Ice used for chilling water of barrels can be contaminated by cold water. The following actions are recommended in order to preventing from transmission of different agents through

water or ice especially in warm seasons.

1. Utilization of bottled water, without any contamination specially for producing ice (there should be no connection between water and sewage). Municipal water samples revealed no or insufficient chlorination and fecal coliform bacteria were detected in samples from 6 of 10 wells tested (8).
2. Electrical reservoirs system can be used for chilling water instead of ice. There is usually no contact between water in these reservoirs and the environment. This method has been started in a few regions of Mashad city and we hope to use this system everywhere.
3. Carring ice in specific machines in a way that is safe (no contact with dust and side of gutters during transportation).
4. Ice bars can be packaged with nylon in the factory before offering to the market in a way that there is no contact with ice surface and the hands of the worker. Ice bars should be placed in specific plastic containers.
5. Avoid ice kept in reservoirs by the streets vendors.
6. Use of gloves during transportation can be helpful.

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